Acta Genetica et Statistica Medica

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GUNNAR DAHLBERG

IN MEMORIAM

The founder and editor of this journal, Professor Gunnar Dahlberg, died in Uppsala on July 25, 1956. Born August 22, 1893, Gunnar Dahlberg received his medical education at the University of Uppsala and became a licensed physician in 1920. He devoted only a short time to the practice of medicine, becoming interested very early in genetics and statistics. In 1921 the Swedish State Institute for Human Genetics was founded and

Dahlberg was one of the first research assistants of this Institute. In 1926 he published his first comprehensive scientific work "Twin births and twins from a hereditary point of view" for which he received the degree of Doctor of Medicine with full honours from the University of Uppsala. This monograph, which is still one of the corner stones of modern twin research, soon gave him a place among the leading human geneticists.

The same year Dahlberg became docent (associate professor) of human genetics and medical statistics at the University of Uppsala. In 1936 Dahlberg succeeded Professor Lundborg as director of the State Institute for Human Genetics and as Professor of Human Genetics at the University of Uppsala, a position which he held until July 1, 1956, when he retired because of ill health.

The unusual width of Dahlberg's scientific interests and endeavours is documented by his publications, of which a list has been recently published in this journal (vol. 4, no. 2/3). To the readers of this journal, Dahlberg should be known mostly because of his contributions to theoretical human genetics and especially population genetics. The quality of his work requires no comment here. He received many honors and was awarded an honorary doctorate of law by the University of Aberdeen as well as an honorary doctorate of odontology by the Royal Dental School in Stockholm.

When Hitler came into power in Germany and the nazis began to interfere with science, especially abusing the new science of human genetics, Gunnar Dahlberg clearly foresaw the consequences and stood up to fight for scientific honesty. His book "Race, Reason and Rubbish" (Allen and Unwin, London 1942) exposed the nazi crime on human genetics. However, this book could still be recommended to many parties of 1956 who entertain outmoded ideas on human races. Gunnar Dahlberg's fight against dictatorship was not only carried out from behind a desk. He took an active part in aiding many refugees during and after the war. For his activities during the war, he received from Great Britain the King's Medal for Service in the Cause of Freedom. I believe that he was more proud of this award than of any of his scientific honours. Later, Dahlberg took the same attitude against the interference of the communists with genetics and the freedom of science. He resented dictatorship in whatever external appearance.

The personal destiny of Professor Dahlberg was a hard one. In 1945, only 9 years after his appointment as director of the Institute, he suffered a stroke which resulted in a rightsided hemiplegia and severe impairment of his speech. The fact that he was back in office only two months after the incident bears evidence to his remarkable energy and self-control. In spite of his severe physical handicap, he was able to continue his work at the Institute successfully until 1955.

Gunnar Dahlberg had a great influence on the development of scientific medical research in Uppsala. It is well known that many clinicians entertain a certain horror of statistics and this was much more pronounced 30 years ago. Professor Dahlberg did much to alleviate this horror and never failed to aid in planning research projects or to help with statistical analyses of a great variety of data. The last medical monograph for which he was an active adviser in scientific method and statistics bears the number 119.

Gunnar Dahlberg was an unusual man with a broad knowledge in science as well as in arts. His main interest was centered on the application of rigid statistical methods to various problems in human genetics. I can do no better than closing this obituary by quoting the following lines of Francis Galton. They represent something Gunnar Dahlberg lived up to.

"General impressions are never to be trusted. Unfortunately when they are of long standing they become fixed rules of life, and assume a prescriptive right not to be questioned. Consequently those who are not accustomed to original inquiry entertain a hatred and a horror of statistics. They cannot endure the idea of submitting their sacred impressions to cold-blooded verification. But it is the triumph of scientific men to rise superior to such superstitions, to desire tests by which the value of beliefs may be ascertained, and to feel sufficiently masters of themselves to discard contemptuously whatever may be found untrue."

Jan A. Böök, Uppsala

THE FIRST INTERNATIONAL CONGRESS OF HUMAN GENETICS

Copenhagen, August 1–6, 1956

PROCEEDINGS

PART I

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At the business meeting on August 4., 1956, it was unanimously decided that The Second International Congress of Human Genetics is to be held probably in 1961.

A provisional committee was elected to prepare this Congress and to investigate the advisability of establishing an International Organization for Human Genetics.

The members of the provisional committee were elected as follows:

FRANCESCHETTI, A.	Geneva
KALLMANN, F.J.	New York
KEMP, T.	Copenhagen
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Minnesota Human Genetics League

Address at the Opening of

THE FIRST INTERNATIONAL CONGRESS OF HUMAN GENETICS

August 1st, 1956

By Julius Bomholt
Danish Minister of Education

Mr. President, Ladies and Gentlemen.

It is a great pleasure and honour to me, on behalf of the Danish Government, to bid you all a hearty welcome to this First International Congress of Human Genetics.

The years after the Second World War have to an ever increasing extent been characterized by international congresses: the world has become smaller, and with the rapid development of the means of communication in our time it only takes a few days for research-workers from all parts of the globe to get together for the discussion of the present day's great scientific problems, of which many can only be solved really effectively by team-work extending across all national frontiers and the seven seas.

Human genetics and its importance in preventing the occurrence and spread of hereditary diseases have just now become of intense topical interest by the perspective arising from the peaceful use of atomic energy and have made this Congress and its subsequent results particularly momentous.

Man's first actual encounter with atomic energy about 11 years ago by now, was a terrific shock, and in the minds of all citizens of countries of all the world a profound anxiety arose concerning the future use of this new devastating force.

It is the greatest international task of our time to secure that this force shall not in future be released for the destruction of mankind.

But at the same time it has proved that this new source of energy utilized in the service of peaceful purposes may mean a revolution of all our technical development, and at a single blow remove all concern about the sharply dwindling supplies of our globe of the sources of energy most frequently used so far—coal and oil. I feel convinced that we are standing on the threshold of a development which our imagination can hardly grasp—and we in my generation have after all experienced a development which you would not think could be surpassed (motor cars, aircraft, radio, T.V. etc.).

This development raises no end of problems, partly on an international level, partly to the government of the individual countries—and particularly to the small countries as Denmark; in fact the Government of our country is at present intensely occupied with these questions.

But the technicians' continued endeavours to use atomic energy in the service of peaceful purposes have caused that with increasing vigour voices have been raised in warning by geneticists and biologists calling attention to the grave responsibility undertaken—particularly towards the coming generations—by releasing these im-

mense latent forces and exposing the human body to a radiation whose consequences cannot be fully foreseen.

We owe a debt of profound gratitude to the research-workers who have raised these problems. Their initiative has already resulted in keen watchfulness which cannot be keen enough. We now pin our faith on the ability of further research to combat and eliminate the risk that may exist, so that we may enter this new epoch with confidence, also with respect to the coming generations.

The great importance attached to the discussions of the coming days is also seen from the fact that the United Nations have asked to be informed of the results of the Congress and that the World Health Organization has convened a session of experts here in Copenhagen, in direct continuation of the Congress for the discussion of its results.

Wishing you a series of fruitful discourses which may form the basis of a further extension of international cooperation in these fields, I hereby declare

The First International Congress of Human Genetics open.

Address at the Opening of

THE FIRST INTERNATIONAL CONGRESS OF HUMAN GENETICS

August 1st, 1956

By Tage Kemp

President of the Congress

Ladies and Gentlemen, Colleagues and Friends.

On behalf of the Danish organizing committee of The First International Congress of Human Genetics *it is my privilege and honour to welcome you all heartily to* The First International Congress of Human Genetics *in Copenhagen.*

Within recent years very much attention has been drawn to the dangers which our load of mutations involves for the human race, the risk of reduced fitness and even the perils of genetic death have been strongly emphasized.

These dangers could, however, be considered from another point of view. Human beings possess a treasure of normal genes and a heritage of valuable hereditary factors.

It is the task and responsibility of mankind in our generation, and in particular of the students of human and medical genetics to protect this treasure and to shelter this heritage from harmful influences and threatening hazards.

Beyond a definite intensity further increase in radiation presents a potential danger to the human race as well as to plant and animal life. The most serious and effective precautions to control and to prevent this risk and this danger must be taken.

On the other hand the danger must not be over-estimated, and unnecessary anxiety ought to be avoided.

This is why the study of hereditary lesions is of such great consequence. The knowledge of the conditions affected by heredity makes it possible to follow and control their development and fluctuation in the population and to ascertain the behaviour of hereditary diseases down through the ages.

An increasing social consciousness has been manifesting itself, in democratic countries during recent years, a growing feeling that society must make living conditions tolerable for everybody. At the same time, democratic social conditions prevent the misuse of genetic-hygienic measures and ensure that due consideration is paid to personal liberty.

The rise and rapid progress during the past 50 years of human genetics and its subdivisions, such as medical and clinical genetics as well as radiation genetics, population genetics and immunogenetics, has added largely to our knowledge of this subject. The first attempts have been made to organize an epidemiological control of hereditary diseases, and a medicogenetic registration has recently been established in some countries.

At the same time, genetic counselling is being practised more and more extensively within all branches of medicine.

If this development continues, the time is drawing near when man can control his own biological evolution and also command his environments and conditions of life to an increasing extent.

Medical genetics in connection with the associated advice and registration create the necessary foundation for measures aiming to prevent hereditary diseases. Consequently the time has come to intensify international collaboration within the vast field of human genetics.

As a step in that direction The First International Congress of Human Genetics *is being held in Copenhagen to promote the progress of human genetics, thereby to create new possibilities for improving the health and happiness of man.*

From more than 30 countries from all parts of the world the students of human genetics have crossed oceans and continents and have overcome the tediousness of the long travel, in order to attend the congress in Copenhagen.

It is our sincere hope that during our meetings we shall by our combined effort be able to elucidate the problems of hereditary characters in man, and to solve some of the riddles within the field of human inheritance.

With this wish, the Danish organizing committee welcomes you most heartily to the congress.

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inside (in brackets): Page-numbers of the Proceedings of the First International Congress of Human Genetics.
outside: Page-numbers of the "Acta Genetica et Statistica Medica".

Bedeutung der Zahlen am Kopf der Seiten:

innen (in Klammern): Seitenzahlen der Proceedings of the First International Congress of Human Genetics.
außen: Seitenzahlen der «Acta Genetica et Statistica Medica».

Signification des chiffres au haut des pages:

à l'angle interne (entre parenthèses): Numéros des pages des Proceedings of the First International Congress of Human Genetics.
à l'angle externe: Numéros des pages de l'«Acta Genetica et Statistica Medica

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PART I

Experimental Pathology and Cytology in Relation to Human Genetics

Heredity in Cancer

Muller, H. J.: Acta genet. *6*, 157–168, 1956

Department of Zoology, Indiana University, Bloomington, Indiana, U.S.A.

FURTHER STUDIES BEARING ON THE LOAD OF MUTATIONS IN MAN[1]

By H. J. MULLER

1. Mutation frequency at specific loci

One approach to the problem of mutational damage in man lies in the studies of the frequencies with which highly damaging mutants of specific types occur. We may leave out of account here the studies of autosomal recessives because their frequencies are too much influenced by extremely slight, indeterminable amounts of selective disadvantage or advantage when heterozygous. As for the dominants, there is the major objection that any given phenotype might be produced by the mutation of any one of a number of different loci, lying in quite different chromosome regions. Even a whole syndrome may be subject to such multilocus origination, as is true of the Minute-bristle syndrome in Drosophila and on a lesser scale of other mutants in that species. However, there are more types of Drosophila dominants that appear to be unique in locus. This difficulty (as well as that of selective survival of the heterozygote) is largely avoided in the case of sex-linked mutants, and it is therefore notable that these, as represented by hemophilia and muscular dystrophy (*Duchenne*), give estimated mutation rates not lower but even slightly higher than is usual for autosomal dominants.

An objection recently emphasized by *Vogel* [1954] is that some cases may represent somatic mutation or "phenocopies". He estimates from data on transmissibility that about ¾ of sporadic retinoblastoma cases are of this nature and that the true mutation rate here is about 1 in 230,000.

[1] Zoology Department contribution No. 630. This study was supported by a grant from the U.S. Atomic Energy Comission (Contract AT [11–1]–195) and from the American Cancer Society.

This source of error does not seem to have been so serious in the other cases concerning which evidence is available.

Finally, it has been objected that mutants with a higher mutation rate have *ipso-facto* drawn attention to themselves and therefore been chosen for study, with a resultant bias in favour of higher values. This difficulty also is a very real one.

If these objections applied to a serious degree an extremely high variation, ranging over several orders of magnitude, would be expected in the frequencies of mutation already estimated. Moreover, the cases with lower frequencies, representing on the whole the single-region, unselected types, without phenocopies, would vary less from one another than the rest do, tending to form a cluster. The fact that the whole range of some dozen mutants with reasonably well determined frequencies occupies only an order of magnitude, without predominant clustering at the lower frequencies (see for instance *Haldane* [1949], *Neel* and *Falls* [1951]), therefore argues against these objections, as does also the fact that the types most reliable as regards locus, namely, the sex linked mutants, are not the ones with the low frequencies. Of course, even if we accept the figure of 1 to 2 in 100,000, about which this range centers, as being representative, it must still be admitted that more than one sub-locus may be concerned in any given case: that is, that we may be dealing with a series of pseudo-alleles rather than strict alleles. However, this will not invalidate the use of such data as material for the further calculation of total mutation frequencies, as will be explained later.

In view of these difficulties it is fortunate that *Russell* has by this time obtained results of value on the mice that he used as controls to his irradiation experiments. Although the details have not been published, the individual mutations and the total numbers among which they have been found were listed by *Russell* in his address at the Mutation Symposium held at Brookhaven in June, 1955. From this report, as well as from personal communications, it appears that the over-all spontaneous mutation rate per allele or pseudoallele series averaged about 1 in 100,000. It should be recalled that in this work recessive "visible" alleles, arising in the male, of mutants of seven different loci were looked for by crossing normal males to females containing the recessive genes (usually in heterozygous condition). The scale of the work, involving the examination of a number of F_1 mice of the order of 200,000, allowed the detection of about 8 mutants of independent origin. This was not enough to establish frequency differences from locus to locus, except that it made probable a somewhat higher frequency for the S locus, which was also found to have a

higher induced frequency. But the data did provide an over-all per-locus rate, and that rate is about 1 in 100,000, with an error of less than a factor of two in either direction, if mutations occurring early in the germ line and thus giving clusters of mutants are omitted as having too large a statistical error. If they are included the rate is distinctly higher. However, in Drosophila, at least, it was found in large-scale work on lethals (*Muller* [1946, and unpublished]) that such omission does not reduce the calculated rate by more than some 10 to 20 per cent. Thus 1 in 100,000 may be considered a kind of basal value.

The agreement between the results obtained by the studies on man and the basal value of 1 in 100,000 in the mouse is surprisingly close and should help to give us confidence in the figure for man. In this connection, it should be taken into account that, other things being equal, a lower rate is to be expected from the mouse than from man both because of the mouse's 30 times shorter duration of reproductive life, and his far smaller number (probably about half as many) of cell divisions in the germ track. A number of considerations, which time does not permit examination of here, indicate that the mutation rate would be more nearly proportional to number of cell divisions than to time. Thus these results in mice would be in best agreement with a per-locus or allele-series rate of 2 in 100,000.

The objection that in the mouse also there was a selection of loci with higher rates cannot be ruled out entirely. However, it is to be noted that the recessives here used were consciously chosen only for their convenience in regard to classifiability, viability and fertility, and that mouse breeders have for many years, in large-scale breeding, been on the watch for mutations, and have sought to establish stocks of even the rarest of those that were good in these respects: in fact they were probably most zealous in breeding the rarest ones. In view of this it would be strange if the mutation rate observed in the mouse should represent a marked selection for high frequency and if, in addition, the human data should represent the same kind of selection to just such a degree as to result in the remarkable agreement found. We conclude, then, that the value of 1 to 2 in 100,000 for the per-locus or allele-series rate in man is probably well founded, and that, of these two alternatives, the upper one, 2 in 100,000, is the more likely value.

2. The relation of specific-locus mutation rate to total load

To convert this so-called per-locus figure into one approximating the *total* rate of detrimental mutations we must multiply it by the ratio

that all detrimentals bear to those of one allele series. It is true that this ratio must be derived from Drosophila, which is a very long way indeed from man, and distinctly simpler in its general morphological and physiological pattern as well as possessed of much less desoxyribonucleic acid per chromosome set. There is no reason to suspect that the individual genes, in the sense of chromosome regions serving as the basis of allele and pseudo-allele series, are simpler in Drosophila than in man, but it does seem likely that the chromosome set as a whole is less compound in Drosophila, in the sense of containing fewer such functional parts, and it certainly would be strange if the genotype of Drosophila were *more* compound. If we admit this argument, we must conclude that the ratio of all detrimental mutations to mutations of single "visible" allele-series is not higher in Drosophila than in man and is perhaps a good deal lower. Therefore the figure obtained by multiplying the human per-locus frequency by the Drosophila ratio would give only a minimum estimate, perhaps a very low minimum, of the total rate of detrimental mutations in man. Another reason why the Drosophila estimate is minimal for man is that it is minimal even for Drosophila. For it takes into account only mutations that rather distinctly reduce survival prior to maturity. It does not include mutations whose effects on the survival of the immature individual are small (say, less than 10 per cent), or those whose effects are exerted only on later stages or only on fecundity. If those other effects had been included the ratio might for all we know have been as much as double the estimated figure.

The ratio of total detrimental to allelic mutations thus far detected in Drosophila is arrived at by several steps, utilizing any one of a number of different pathways. But the figure thus obtained is in any case about 10,000, or somewhat more. As an example, we may note that in certain studies on spontaneous mutation in our laboratory (*Muller, Valencia* and *Valencia* [1949]) the sex-linked lethal rate in the male bore a ratio of about 350 to the "specific-locus visible" rate. The sex-linked lethal rate in turn is known to be about a sixth of the total lethal rate, so that the ratio of all lethals to mutations of a given visible allele series is about 6×350 or 2100. Other work has given a slightly higher ratio. Furthermore, work by myself and by *Kerkis* working under my direction (*Muller* [1928, 1934], *Kerkis* [1935, 1938]), and independent work by *Timoféeff-Ressovsky* [1934, 1935] has shown that the ratio of X-ray-induced detrimental (including lethal) mutations, having effects distinct enough for detection, to X-ray-induced lethals, is about 4 to 1. Multiplying 4 by 2100 we obtain 8400 as the ratio of total detected detrimental mutations to per-locus

mutations. However, the ratio of detrimentals to lethals must certainly be higher among spontaneous than among X-ray-induced mutations since ionizing radiation through its relatively high production of chromosome aberrations results in many more cases of position effects and deficiencies, in comparison with "point mutations", than arise spontaneously, and these changes are known to include a much higer proportion of lethals than the point mutations do. For this reason too, then, 10,000 is to be regarded as a minimum figure for the ratio of detrimental to "per-locus visible" mutations, even in Drosophila (*Muller* [1955a]).

It is to be noted that this figure need not be understood as the number of genes but only as the empirical ratio of all detrimental mutations thus detected to mutations of one average allele series, and that pseudo-alleles are here included under this designation (*Muller* [1955b]). Thus the terms "locus" and "per-locus" in this connection are only abbreviations.

When, now, we multiply the "per-locus" figure of 1 to 2 in 100,000 for man by 10,000 we obtain the value 1 to 2 in 10, that is, one- to two-tenths, as the minimum frequency of gametes in man that contain a newly arisen mutation, and twice this, or two- to four-tenths, as the minimum frequency of new mutations per diploid individual. In a population having a stable over-all frequency of mutant genes (a condition that must approximately hold over any long period of time) there must be about as many mutant genes eliminated by "genetic death" of the individuals containing them, in consequence of their disabilities, as the number arising anew by mutation, i.e. at least two to four mutant genes in excess of the average load for every ten individuals of the population.

To a certain extent these genes will be eliminated through some concerted action, either on the part of alleles that have come together to form a homozygous recessive, or of two or more genes at different loci that act in some degree synergistically. This will cause the number of *individuals* that undergo genetic death to be somewhat lower than that of eliminated genes, i.e. lower than twice the mutation rate (even after allowance has been made for the frequency of individuals having more than one mutant gene in excess of the average). There are, however, several grounds for inferring that such synergism is not prevalent enough to reduce the elimination rate of individuals very much below that of the mutant genes themselves. If that is true, then to match the minimum mutation rate of two to four individuals in every ten there must usually be a genetic elimination rate, through premature death or failure to reproduce, of nearly as high a proportion.

This risk of genetic death, of nearly .2 to .4, is shared in greater or lesser degree by virtually all individuals of the population, inasmuch as all of them contain several or many detrimental mutations. Therefore this figure can also be considered as expressing the amount of "loss of fitness", to use *Haldane*'s [1937] term, of the average individual: his lesser biological potency as compared with that of a hypothetical man having no detrimental mutant genes. How much of tangible infirmity and infelicity this much risk of genetic elimination usually entails is a matter that can best be estimated by physicians on the basis of carefully collated experience.

3. The load as disclosed by inbreeding

A different approach to the problem of how much mutational load has been accumulated in human populations is through studies of the effects of inbreeding. One application of this method has recently been used by *Slatis* [1954] in deriving a tentative figure (8) for the frequency of recessive genes causing definitely distinguishable rare abnormalities. We are here interested, however, in a more general view, that will bring us closer to an estimation of the total mutational load.

The present writer made a start at such an approach some fifteen years ago, on coming across a study published in 1908 by *G. B. L. Arner*, entitled "Consanguineous Marriages in the American Population". In this monograph data were obtained from genealogies that dealt, among other things, with the survival up to 20 years of age of 672 children resulting from first-cousin marriages and that of 3184 children, of the same genealogies, resulting from marriages between non-relatives. The former group showed 83.2 per cent survival and the latter 88.4 per cent, the difference being highly significant. Dividing the former figure by the latter, it turns out that only 94.1 per cent of those who should have survived if the parents had not been inbred were able to survive if their parents were first cousins. That is, the inbreeding resulted in the death, before 20 years of age, of about 6 per cent of those who would otherwise have lived. Since, however, first-cousin inbreeding gives only a $^1/_{16}$ probability of homozygosis of genes that would otherwise have been heterozygous, we may multiply this 6 per cent mortality by 16. This gives us 96 per cent as the sum of the excess risks of genetic death (as compared with non-inbred individuals) that would on the average be undergone between birth and twenty years by hypothetical individuals that were homozygous for all the genes contained in just one of the gametes that produced them.

It was this calculation that led the present writer to say, at a symposium on genetics and public health held in 1947 (published 1948): "a calculation... from results of inbreeding in man... leads to the conclusion that every person on the average contains heterozygously at least one lethal gene or group of genes which (homozygously would) ... kill an individual between birth and maturity." Actually, the above data indicate the possession by each individual of two such groups of genes, heterozygously, since he is derived from two gametes, each containing one such group. That the lower value given in the statement, which was intended to be a minimum estimate, probably made much more allowance than necessary for statistical and other errors has been indicated by estimates recently made, in part independently, by *Morton* and *Crow*, on the basis of other data (see *Morton*, *Crow* and *Muller* [1956]). Moreover, judging by the frequency of deaths at other periods of life than that here considered—deaths that nevertheless play a role in genetic survival,—and judging also by the frequency of genetically influenced infecundity, the effects noted in *Arner*'s study probably represent not more than half of the total number of "lethal equivalents" (to use *Morton* and *Crow*'s term). Thus the indicated number of these lethal equivalents per diploid individual is probably at least 4. It should be emphasized that this does not mean four actual lethals, but a collection of slightly detrimental genes and of some lethals, all of which would, if dispersed among different individuals in homozygous condition, tend to give a total of four genetic eliminations.

4. The derivation of mutation rate from total load

Crow (*Morton*, *Crow* and *Muller*, *ibid.*) has found a relatively simple way of translating this accumulated load into terms of mutation frequency, provided that the assumption is accepted that the great majority of mutant genes have a certain low degree of dominance. Direct evidence that most mutant genes in man have some dominance was long ago given in studies of *Levit* [1936] that have recently received support in work of others. Grounds for inferring that the same principle applies in Drosophila and other organisms have been adduced by the present writer [1950a, b, c] on consideration of the mode of expression of deficiencies and duplications, the facts of dosage compensation, and special studies of the expression of "visible" mutants when heterozygous. Further both *Stern et al.* [1948, 1952] and the present writer in collaboration with *Campbell* (*Muller*, *ibid.*), in independent work, have found that lethal

and near-lethal genes in Drosophila have, on the average, some 4 to 5 per cent of dominance.

Some indirect evidence that detrimental mutant genes in man have an amount of dominance of this same order is to be found in the similarity in the frequency of specific genes for detrimental recessive abnormalities in Japanese and European-derived populations (as found by *Komai* [1947, 1956], and by *Neel et al.* [1949]). For, as compared with modern Europe and America, the amount of inbreeding in Japan is exceedingly high and probably has been so for millenia, resulting, we may calculate, in at least ½ per cent of homozygosity due to this cause in Japan and very likely 1 per cent (i.e. the coefficient of inbreeding is .005 to .01). In consequence of this very high degree of inbreeding, mutant genes in the Japanese population would have been eliminated much faster than in Europe and would have attained distinctly lower frequencies than in Europe, unless one of the following conditions had held. (1) If the dominance of the so-called recessive mutants were usually distinctly more than 1 per cent, so as to constitute the overriding cause of their elimination; or (2) if the amount of inbreeding in medieval and ancient Europe was (as some indications suggest[1]) about as high as in Japan.

There is to be sure a third possibility, namely, that there is a much lower mutation rate in Europe, which compensates for the supposed lesser rate of elimination via homozygotes. This, however, would be a purely *ad hoc* assumption of very questionable character, and we shall ignore it here. If the first possibility and not the second is correct (although it should be noted that they are not mutually exclusive) it would follow that the usual dominance in man should be taken as at least 2 per cent, to mask the difference in inbreeding, and it might well be a good deal higher. We should postpone until later a consideration of the consequences of the second possibility.

Now the method of conversion of our figure for lethal equivalents —4 or more—into one for mutation rate, when the amount of dominance is given and when it overrides the effects of homozygosity, may be explained as follows. We have seen that any given mutant gene tends to persist in the population until it causes one elimination (if we neglect the overlapping of eliminations). This elimination, if the dominance is as great as we have inferred, usually takes place in the heterozygote. Thus the frequency of eliminations, and the associated "damage" or "loss of

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[1] I am indebted to Dr. *W. Lenz* for having called my attention to the likelihood of this possibility.

fitness" in the population, caused by detrimental mutations arising at any given locus or chromosome region, is about equal to the frequency of mutations at that locus or region that arise in germ cells of both sexes; that is, it is about twice the mutation rate. And the *total* frequency of eliminations or total amount of genetic damage, either in the population as a whole or in the average individual is, roughly, twice the total rate at which detrimental mutant genes are arising. In other words, the 4 or more lethal equivalents contained by the average person, that would be sufficient to kill 4 times over if they were made homozygous, actually exert in their usual heterozygous condition a tendency to genetic death equal to 2μ (where μ is the mutation rate). Assuming, as an approximation sufficiently close at this stage, that they are all heterozygous and that, to be conservative, their dominance (that is, their degree of expression in heterozygous as compared with homozygous condition) is only 2 per cent, it is evident that in the ordinary individual this load is bringing about an amount of damage or risk of elimination of $4 \times .02$, or 8 per cent. Moreover, since as we have just seen this is approximately twice the mutation rate per gamete, μ would be .04. If, however, dominance should be taken as 5 per cent μ would be .1. In either case the result is in satisfactory agreement, considering the uncertainties involved, with the figure of .1 for μ arrived at by our first, entirely different method.

The details of the calculations, together with various qualifications, subsidiary considerations, and mathematical relations, are being set forth elsewhere (*Morton*, *Crow* and *Muller*, *ibid.*). It may be noted here that, as *Crow* has pointed out, the value for dominance to be used in this calculation should be the harmonic mean value, since the persistence of a gene is reciprocally related to its dominance, and that the harmonic mean value indicated by certain not too extensive Drosophila data suitable for a study of this question (*Muller* and *Campbell*, unpublished) has turned out to be about 2 per cent, although with a relatively large error, whereas the arithmetic mean was 4.4 per cent. There are, however, reasons for suspecting that the value may be higher for the more numerous less detrimental genes, and that it may be higher for man than for Drosophila. This would tend to make our estimate of mutation rate based on this method, like that on the other one, a conservative estimate. On the other hand, the true value could not be inordinately higher than on this estimate. For if it were the rate of elimination would have to be higher than could be tolerated by an ordinary human population, with its relatively low rate of reproduction.

We will now return to consider the consequences of the possibility

that in ancient and medieval Europe, as in Japan, the frequency of homozygosity caused by inbreeding was .01 or more. In that case, on the very extreme postulate that the dominance of mutant genes is practically zero, the estimated 4 lethal equivalents of the usual individual would attain, on the average, one per cent of expression through inbreeding (and a much lesser expression through the meeting of independently arisen alleles). Hence in this case the elimination rate, the loss of fitness, and the mutation frequency, would be $4 \times .01$, that is, .04, a value like that obtained on the assumption of a 2 per cent harmonic-mean dominance. If, however, this or a greater degree of dominance existed at the same time, which is very likely to have been the case, these values would rise to nearly .1.

5. Conclusions and Prospectives

We may conclude that modern studies, through two independent routes, support the conclusion that the frequency of detrimental mutations in man is of the order of one in ten gametes, with a present factor of uncertainty of about 3 in either direction. That is, the rate is of the order of magnitude centering, geometrically, about one tenth. The elimination rate and the loss of fitness, under equilibrium conditions, would probably be somewhat less than twice as great as the mutation rate.

It is to be expected that further studies along these lines will provide qualitative detail and greater exactitude. Among questions on which light will be thrown by application of the second method, based on consanguinity differences, are the following. (1) The extent to which detrimental mutations are eliminated as heterozygotes, as shown by a comparison of their frequencies, individually or *en masse* (e.g. through mortality studies), in populations that have long been subjected to very different degrees of inbreeding. (2) The frequency of so-called "overdominant" mutants (those which although detrimental when homozygous are superior to normal when heterozygous). (3.) The extent of synergism between non-allelic mutants in the causation of damage and elimination, as judged by the degree of upward curvature, rather than linearity, of the graph relating mortality and other damage to the closeness of inbreeding, when results from unusually close inbreeding are included. (4.) The extent to which differences in mortality, infecundity, etc., are in general genetic, i.e. selectable, as found by comparison of the mortality, infecundity, etc., of the offspring of non-relatives with the value for the genetic contingent of these quantities as deduced from the results for homozygotes taken in connection with determinations of dominance. (5.)

The extent to which our modern civilization is decreasing genetic elimination below the equilibrium level. This would be evidenced by the differences in mortality, infecundity, etc., resulting from inbreeding, between populations subjected to different present standards of living and medical care. Further evidence would be obtained by a study of the differences between the calculated genetically occasioned contingent of the mortality, infecundity etc., among the offspring of non-relatives in the compared populations. For this purpose it is imperative that data of these kinds be obtained from populations still living under primitive conditions of selection, before it is too late. (6.) The amount of increase in mutation rate that a population under given conditions of living can tolerate without serious or continued genetic deterioration or, alternatively, the rapidity, duration, and quality of deterioration that a given increase in mutation rate would cause. In this connection it may be noted that knowledge of the total spontaneous mutation rate and load, whether obtained by this or any other method, is an invaluable means of assessing the total effect that would be produced by a given amount of radiation or other mutagenic agent, if an index of the mutagen's effectiveness had been obtained by data on mutations at specific loci or of some otherwise specified category.

REFERENCES

Arner, G. B. L.: Consanguineous marriages in the American population. Stud. Hist. Econ. Publ. Law. Columbia Univ. *31*, 3, 1–100, 1908.

Haldane, J. B. S.: The effect of variation on Fitness. Nature *71*, 337–349, 1937.

— The rate of mutation in human genes. Proc. 8th Int. Congr. Genet., 1948, Hereditas *35* (Supp.), 267–273, 1949.

Kerkis, J. J.: The preponderance of "physiological mutations". Summ. Commun. XV. Int. Physiol. Congr. (Leningr.-Mosc.), pp. 198–200, 1935.

— The frequency of mutations affecting viability. Izv. Akad. Nauk. (Otd. mat.-est., Ser. biol.) 75–96, 1938. (Russian and English text.)

Komai, T.: Pedigrees of hereditary diseases and abnormalities found in the Japanese race (1934–1947). Japan Soc. Prom. Sc. Res. 4th Spec. Com. Report 2. 225 pp. 1947.

— Genetics of Japan, past and present. Science *123*, 823–826, 1956.

Levit, S. G.: The problem of dominance in man. J. Genet. *33*, 411–434, 1936.

Morton, N. E., J. F. Crow and *H. J. Muller:* An estimate of the mutational load in man from data on consanguineous marriages. Proc. Nat. Acad. Sci., Wash. *42*, 1956.

Muller, H. J.: The problem of genetic modification. Z. indukt. Abstamm., Suppl. I. 234–260, 1928.

— Radiation genetics. Proc. IV. Int. Radiol. Kongr., Zürich *2*, 100–102, 1934.

— Age in relation to the frequency of spontaneous mutations in Drosophila. Yrbk. amer. philos. Soc. for 1945, 150–153, 1945.

— Mutational prophylaxis. Bull. N. Y. Acad. Med. *24*, 447–469, 1948.

— Evidence of the precision of genetic adaptation. Harvey Lect. *43*, 165–229, 1950a.
— Partial dominance in relation to the need for studying induced mutations individually. J. cell. comp. Physiol. *35*, 205–210, 1950b.
— Our load of mutations. Amer. J. hum. Genet. *2*, 111–176, 1950c.
— How radiation changes the genetic constitution. Bull. atomic Scient. *11*, 329–339, 1955a.
— Comments on the genetic effects of radiation on human populations. J. Hered. *46*, 199–200, 1955b.
Muller, H. J., J. I. Valencia, and *R. M. Valencia:* The frequency of spontaneous mutations at individual loci in Drosophila. Genetics *35*, 125–126, 1949.
Neel, J. V. and *H. F. Falls:* The rate of mutation of the genes responsible for retinoblastoma in man. Science *144*, 419–422, 1951.
Neel, J. V., M. Kodani, R. Brewer and *R. C. Anderson:* The incidence of consanguineous matings in Japan. Amer. J. hum. Genet. *1*, 156–178, 1949.
Russell, W. L.: The mutational characteristics of specific loci. Lecture before Mutation Symposium at Brookhaven Nat. Lab., N.Y., June 17, 1955 (Unpub.), title in Brookhaven Symp. in Biol. *8* (Mutation), p. ix, 1956.
— Genetic effects of radiation in mice and their bearing on the estimation of human hazards. Peaceful Uses of Atomic Energy, U. N. *11*, 382–383, 1956.
Slatis, H. M.: A method of estimating the frequency of abnormal recessive genes in man. Amer. J. hum. Genet. *6*, 412–418, 1954.
Stern, C. and *E. Novitski:* The viability of individuals heterozygous for recessive lethals. Science *108*, 538–539, 1948.
Stern, C., G. Carson, M. Kinst, E. Novitski and *D. Uphoff:* The viability of heterozygotes for lethals. Genetics *37*, 413–449, 1952.
Timoféeff-Ressovsky, N. W.: Auslösung von Vitalitätsmutationen durch Röntgenbestrahlung bei Drosophila melanogaster. Strahlentherapie *51*, 658–663, 1934.
— Auslösung von Vitalitätsmutationen durch Röntgenbestrahlung bei Drosophila melanogaster. Nachr. Ges. Wiss. Göttingen (Math.-phys. Kl., Biol.) N. F. *1*, 163–180, 1935.
Vogel, F.: Über Genetik und Mutationsrate des Retinoblastoms (Glioma retinae). Z. KonstLehre *32*, 308–336, 1954.

Penrose, L. S.: Acta genet. 6, 169–182, 1956

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MUTATION IN MAN

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1. Introduction

The study of gene mutation in man has two aspects. The first concerns the ascertainment of spontaneous mutation rates at specified loci. This gives information about human evolution in general as well as about the causation of certain rare diseases and defects. The second aspect, which has only recently become significant, concerns artificially produced mutagens and, in particular, the genetical effects of ionizing radiation. In order to estimate the magnitude of these effects a knowledge of spontaneous mutation rates at given loci is required and the sensitivity of these loci to radiation needs to be ascertained.

2. Measurement of spontaneous mutation rate

Estimation of mutation rate in man, in relation to any given hereditary trait, depends upon ascertaining three things, the incidence of the trait in the general population, the nature of the genetical contribution to the cause of the trait, and the fitness of the genotypes concerned. These phenomena are not necessarily constant. As seen in the population at the present time they may not represent the true picture over a long series of generations, during which natural selection has been acting. They only give us the first clue to conditions which govern genic equilibrium in human populations.

There are two standard methods of approach, the direct and the indirect.

(i) *Direct observation*

The most favourable case for estimating mutation rate directly occurs when the gene studied is detectable with certainty or regularity in

heterozygotes. Instances of fresh mutation can then be observed in families where a gene appears in an offspring although it was not present in the parents. The ideal kind of regular dominance required for this is rarely (perhaps never) found in human genetics. Man is a wild species, under natural selection, unlike laboratory stocks, and consequently most single gene effects, especially those shown in heterozygotes, are subject to modification. Even with the most reliable characters, such as blood group antigens, suppression is possible by gene interaction (*Levine* et al. [1955]); such events could easily be misinterpreted as evidence of mutation by the unwary.

The situation for sex-linked genes is quite favourable, theoretically, for direct observation of fresh mutation because modification of a character shown in hemizygous males is usually slight. Occasional families will be observed in which the probability is very great that the disease in the propositus is due to fresh mutation. The proportion of mutant cases can also be inferred if the sibships show an excess of sporadic propositi.

For recessive traits the problem is much more difficult because heterozygous carriers are not detectable in ordinary circumstances. In cases where special techniques have been developed for identification of carriers the problem is resolved into one of detection of mutation for a dominant condition, as demonstrated by *Vanderpitte* et al. [1955] for sickle cell trait. Direct observation of cases of recessive diseases due to fresh mutation is very unlikely to be possible because only a very small proportion of cases of a recessive trait in a given generation can be attributed to fresh mutation in a parent. For diseases in which a single gene is only a part cause and in which environment has a great effect upon manifestation, the contribution of spontaneous mutation is likely to be even less significant. The same applies to conditions due to the interaction of many genes. For none of them can mutation rates be directly determined.

(ii) *The indirect argument*

When the total effects of a gene are very disadvantageous an indirect line of argument can be used for estimating mutation rate, even though the gene may not be manifest in the heterozygous state. Principles on which the indirect estimation of mutation rates can be based were laid down by *Haldane* [1932]. The assumption can be made that the human population is in a state approaching genetical equilibrium under natural selection. It is supposed that disadvantageous genes could not persist in

the population unless their extinction by selective mortality were completely balanced by the recurrence of mutation.

In the case of dominant or sex-linked characters associated with very high mortality, the direct measurement of mutation rate can be supplemented, and its plausibility greatly strengthened, by the indirect argument. The best situation for this combination occurs in the case of a very deleterious dominant trait. This is a rare circumstance. If the disease is not very lethal, there will be difficulty in measuring the unfitness conferred by the gene: if it is very lethal, there is difficulty in proving the dominant mode of inheritance, as it will seldom last even for two generations. Sometimes the problem might be solved for a locus, which had several different known alleles, some producing milder and others severer types of disease. Then, in each of the severest cases, mutation of a lethal allele will be observable. This possibly occurs in both epiloia and chondrodystrophy. For mild alleles, which last for several generations, the proportion of cases due to fresh mutation is correspondingly smaller.

Estimates, which are entirely indirect, are untrustworthy but they have actually been made for a variety of genes recognized only by their recessive effects. One cause of uncertainty with recessive traits is that allowance has to be made for the results of inbreeding. Another likely source of error is that genetical equilibrium can be maintained not only by mutation but also by slightly advantageous effects in heterozygotes. That is to say, on the balance, the total effect of a gene may be much less bad than appears from studying abnormal homozygotes and then an indirect estimate of mutation rate will give much too high a value.

3. Some standard estimates of human mutation rates

Mutation rates have been calculated for quite a large number of genes in man. It is preferable to express them in terms of loci per generation, if we wish to avoid controversy, because slightly different forms of the diseases concerned can be accounted for by the same allele or by different alleles. If there are several very closely linked loci giving rise to a pseudoallelic system, the real mutation rate for each separate element is lowered by a factor depending upon the number of elements in the complex.

(i) *Dominants*

The most exact estimates for supposedly single loci are probably those for very deleterious dominant traits (see Table 1). Allowing for the

probability that more than one disease entity may be classified under each heading, they are maximal values. The average value for six conditions is about 14×10^{-6}.

Table 1. Estimates of spontaneous mutation rates of some human genes: (a) Dominant inheritance; (b) Sex-linked inheritance.

Trait	Mutation rate per million loci per generation	Region	Source[1]	Date
Epiloia (a)	8	England	*Gunther* and *Penrose*	1935
Chondrodystrophy (a)	45	Denmark	*Mørch*	1941
Chondrodystrophy (a)	70	Sweden	*Böök*	1952
Aniridia (a)	5*	Denmark	*Møllenbach*	1947
Microphthalmos without mental defect (a)	5	Sweden	*Sjögren* and *Larsson*	1949
Retinoblastoma (a)	15	England	*Philip* and *Sorsby*	1947
Retinoblastoma (a)	23	U.S.A.	*Neel* and *Falls*	1951
Retinoblastoma (a)	4	Germany	*Vogel*	1954
Partial albinism and deafness (a)	4	Holland	*Waardenburg*	1951
Haemophilia (b)	20	England	*Haldane*	1935
Haemophilia (b)	32	Denmark	*Andreassen*	1943
Haemophilia (b)	27	Switzerland and Denmark	*Vogel*	1955
Pseudohypertrophic muscular dystrophy (b) . . .	95	U.S.A.	*Stephens* and *Tyler*	1951
Pseudohypertrophic muscular dystrophy (b) . .	45	N. Ireland	*Stevenson*	1953
Pseudohypertrophic muscular dystrophy (b) . .	43	England	*Walton*	1955

[1] See *Penrose* [1956a]. * This estimate differs by a factor of 2 from that given by the author but it is based on his material.

Owing the classification of more than one type of chondrodystrophy under the same heading the rate given is likely to be considerably too high. According to *Grebe* [1955] there are several clinical types; and some cases may be due to recessive genes. Furthermore, these different types may have different mutation rates.

Another, dominant, condition, which apparently has a relatively high mutation rate, namely, retinoblastoma, occurs perhaps not infrequently as a phenocopy (*Vogel* [1954]) not transmissible to the next generation. The same idea could be applied also to other conditions listed in Table 1, such as microphthalmos.

The indirect argument, which supports all these estimations, can only be used when there is strong selection against the gene studied. Theoretically it should be possible to obtain mutation figures for several blood antigens, e.g. *ABO* or *MNS*, but selection against any of these genes is too slight and indefinite to be used as indirect support for the mutation hypothesis. On the other hand, the indirect argument can be extended to cover certain cases in which the combination of several genes at different loci is lethal or very deleterious. Thus a lethal condition, caused by the simultaneous presence of two heterozygous genes, will imply that each of the genes concerned mutates frequently enough to make good the loss occasioned when it occurs in conjunction with the other.

Taking all these considerations together we can reasonably assume that the mutation rates for loci giving rise to dominant genes, though somewhat too high, are of the right order of magnitude. It seems that, for most of these dominant diseases, the rate should be considered to be about 5×10^{-6}.

(ii) *Sex-linked loci*

The prevalence in man of sex-linked diseases which are very lethal is difficult to explain except on a mutation hypothesis. Direct evidence based upon observed low incidence of haemophilia in sibships and in maternal collateral relatives also supports this explanation. The matter has been repeatedly investigated by *Haldane* [1946, 1955] and there seems to be some evidence that mutation more commonly occurs in males than in females. The two sex-linked diseases which have given information about human mutation rate are haemophilia and pseudo-hypertrophic muscular dystrophy. In both cases there are many types of illness easily confused with one another clinically. Sex-linked types are identified by pedigree studies and by their occurrence in males only but, by this process, some autosomal cases may occasionally be incorrectly included. A characteristic difficulty is the exclusion of autosomal sex-limited conditions.

In the standard examples of haemophilia and sex-linked muscular dystrophy, mutation rates have been estimated several times but always on the assumption that, in each disease, there is only one locus involved. These rates, as shown in Table 1, are considerably higher than the direct estimates for autosomal dominants. Perhaps the X-chromosome is peculiar in that it has many complex loci or distinct loci with similar effects.

(iii) *Recessive traits*

A recessive trait in man can be defined as one which depends upon a gene in homozygous form. There may be mild manifestations detectable in heterozygotes (e.g. thalassaemia, galactosaemia, cystinuria) but the disease in the homozygote is the effect with which we are concerned. The indirect estimates of mutation rates for recessive diseases, shown in Table 2, assume that the heterozygote is neutral in its effect upon fitness.

Table 2. Indirect estimates of spontaneous mutation rates on the assumption of recessive inheritance.

Trait	Mutation rate per million loci per generation	Region	Source	Date
Juvenile amaurotic idiocy . .	38	Sweden	*Haldane*	1939
Albinism	28	Japan	*Neel* et al.*	1949
Ichthyosis congenita.	11	Japan	*Neel* et al.*	1949
Total colour blindness	28	Japan	*Neel* et al.*	1949
Infantile amaurotic idiocy . .	11	Japan	*Neel* et al.*	1949
Amyotonia congenita	20	Sweden	*Böök**	1952
Epidermolysis bullosa	50	Sweden	*Böök**	1952
Cystic fibrosis of pancreas . .	700	U.S.A.	*Goodman* and *Reed*	1952
Sickle cell anaemia	10000	U.S.A.	*Neel*	1951
Thalassaemia	400	U.S.A.	*Neel*	1951
Spastic diplegia	2000	Sweden	*Böök*	1953
Microcephaly	49	Japan	*Komai* et al.*	1955
Phenylketonuria	25	England	*Penrose**	1956
Schizophrenia	500	England	*Penrose**	1956

* See *Penrose* [1956a].

If the heterozygotes were deleterious, as suggested by *Böök* [1953] for schizophrenia, the values would have to be increased. Conversely, if heterozygotes were slightly favourable, the values would have to be reduced. A very slight amount of heterozygous advantage is sufficient to keep a rare recessive lethal in stable genic equilibrium in the absence of mutation so that the calculation of mutation rate is very easily invalidated. This is an extremely important principle and is worthy of detailed consideration.

Most well known recessive traits cannot easily be supposed to have arrived at their existing levels of gene frequency (e.g. 1/100 for phenylketonuria) by chance or by "drift". The situation for commoner genes is even more striking. For thalassaemia and sickle cell trait (*Neel* [1951]), cystic fibrosis (*Goodman* and *Reed* [1952]), spastic diplegia (*Böök* [1953])

and schizophrenia (*Penrose* [1956a]) improbably high mutation rates have to be postulated. Indeed the maximum rate for sickle cell trait, derived from direct observation on heterozygotes, is much lower than that calculated indirectly (*Vanderpitte* et al. [1955]). These common traits could not have easily established themselves unless the heterozygotes had some advantage. The advantages may have been local ones in the distant past, for example, ability to withstand infections, plagues, famines, abnormal climates and so on.

It is not necessary to postulate any virtue in the heterozygote as such. It could be sufficient if the mutant allele were favourable at one epoch and unfavourable at another epoch, in different circumstances or at different stages of the same life cycle. The principle of genetical stability produced by heterozygous advantage or, more accurately, homozygous disadvantage, is one which has been understood for a long time (*Fisher* [1930]) but only recently taken seriously. In human genetics it is exhibited by such a system as may be present in relation to the sickle cell trait in Africans. The disadvantage of one homozygote, *SS*, which suffers from anaemia, is balanced to some extent by disadvantage of the homozygote, *AA*, which is especially susceptible to malaria caused by *P. Falciparum*. Balanced human genetical systems are shown in metrical traits because the extreme types, which tend to be homozygous, are relatively unfavourable. Examples are stature, birth weight and intelligence level. For intelligence, in particular, there is a marked fertility differential in one direction and a viability differential in the other. That is, low intelligence levels are associated with low viability and high levels with low fertility.

In all such cases of balanced polymorphism the variation, which is apparently reduced in each generation by loss of extreme types, is not maintained by fresh mutation. It is maintained simply because the heterozygotes, who tend to have medium metrical value, are the parents of most children in each successive generation. In these circumstances it is quite useless to attempt to estimate the mutation rates of component genes: any indirect estimate will be far too high.

It has been suggested by *Haldane* [1939] that the converse may be true, namely that mutation rate estimates for recessive traits are often too low. The argument used is that the true incidence, which recurrent mutation would theoretically balance, has in the past been much greater than it is at the present time. This is likely because inbreeding, which facilitates the appearance of recessive diseases, has been gradually diminishing for many decades in all civilized communities. I believe this

argument to be unsound because the incidence of rare recessive traits in man is extremely irregularly distributed. *Tay-Sachs* disease is almost confined to Jewish communities as also is pentosuria, *Cooley*'s disease has centre in the Po delta. Phenylketonuria, on the other hand, does not occur among Jews. Sickle cell anaemia is common in Africans. Juvenile amaurotic idiocy is commonest in Sweden and acatalazaemia has only been found in Japan. These facts suggest that recessive mutations are very rare but that occasionally they have spread for unknown reasons probably connected with heterozygous advantage at one epoch or another. If mutations were not very rare the same set of recessive diseases would appear in all communities, or at least in all inbred communities, throughout the world.

To sum up the discussion on spontaneous mutation rate, my view is that, for a variety of reasons, most mutation rates already calculated are too high; points to be stressed are, first, that mutation may be mimicked by suppression of even the most regular kinds of dominant inheritance, secondly that different conditions are grouped under single clinical headings, and, thirdly, that heterozygotes of established recessive lethal traits are likely to have carried slight advantages in the past even if they do not at the present time.

4. Effect of induced mutations

The immediate effect of an increase above spontaneous mutation rate is most easily calculated when the gene is dominant. The rule, however, is quite general. The increase of incidence of any trait in the first generation, due to induced mutation, depends upon the proportion of cases due to fresh mutation in ordinary circumstances. For lethal dominants and sex-linked traits this proportion is large but in lethal recessives it is very small. It is also small for dominants which are very imperfectly manifested as with those contributing to multifactorial traits. The rule refers to the effect in the first generation or in closely succeeding generations, which especially interest people now living. The total quantitative effect, on the population, of altered mutation rate is theoretically the same whatever the manner of inheritance but, in the case of recessives or heavily modified dominants, a slight effect is maintained over an enormous length of time, many thousands of years.

The proportion of cases of a lethal condition due to fresh mutation in any given generation can be estimated on the basis of the indirect

argument. If the mutation rate, μ, is expressed as a function of the gene frequency thus,

$$\mu = f(q)$$

it follows formally that the proportion of cases in any given generation due to fresh mutation, a quantity which can be called M, is given by the approximation,

$$M = d\mu/dq.$$

For example, for a recessive lethal trait,

$$\mu = q^2, \text{ and so } M = 2q.$$

Substituting 1 40,000 for q^2, the frequency of juvenile amaurotic idiocy as estimated by *Sjögren* [1931], we get M = 1/100. In view of what has been said about the use of the indirect method this is probably an upper limit but it shows how little effect a change in spontaneous mutation rate would have upon the incidence in the next generation after it had occurred or, indeed, in any subsequent generation. Doubling the mutation rate would only increase the incidence by 1 per cent in the first subsequent generation.

5. Sensitivity of human loci to radiation

Much has been written about the probable sensitivity of human loci to radiation using experimental data on lower animals as a basis for comparison. Direct observations on man, however, are essential and three sources of information are at present available.

(i) *The comparison of offspring of selected parents exposed to different quantities of radiation*

This is the method attempted in several comparative studies. Children of radiologists have been examined by *Crow* [1955] and also by *Macht* and *Lawrence* [1955] and the exposed Japanese population by *Neel* and his colleagues (*Neel* and *Schull* [1954]). A development of the same idea is implied in two other proposed types of investigation. One of these is the special examination of children of patients who have received large therapeutic doses of radiation before conception, as may be the case in sufferers from spondylitis. The other suggested method is to examine the incidence of mutations in areas where the natural background radiation is high. Each of these methods, though theoretically possible, has its own special technical difficulties. There is a general objection to all of them,

however. Fresh mutation is a phenomenon which can only very rarely be observed even though it may be occurring all the time. To search for slight increases in incidence of traits which, in the case of known recessives, will not exceed 1 per cent requires the collection of enormous quantities of data and results are certain to be inconclusive. These methods are, in fact, very inefficient even after allowances have been made for sources of error peculiar to each type of enquiry.

(ii) *The examination of parental history in known instances of mutation*

An alternative and more efficient method, which has received scant attention hitherto, is the careful examination of the personal histories of parents and, in certain instances, of grandparents, for groups of cases where fresh mutation is suspected of having played a part in causing disease in the offspring. This method has already produced valuable results by using the simple test of parental age.

Clearly, the older the parent the more likely he is to have been subjected to mutagenic influences. If the influence is background radiation, at the age of 40 the dose will have been twice that received at the age of 20. The net effect on parental age distribution of diseases in the offspring caused by background radiation alone, though definite, would be slight. The expected average increase would be scarcely more than one year above normal parental age (*Penrose* [1955a]). Marked effects confined to one or other parent have, however, been observed in several malformations. Marked increase of father's age has been found in chondrodystrophy and acrocephalosyndactyly, as shown in Table 3. On the other hand, the incidence of mongolism is associated solely with advancing age of mother. It would appear, thus, that, in so far as these traits may have their origins in fresh mutations, the causes must be different. In particular, a marked increase in paternal age strongly suggests some process connected with cell division in the spermatogonial stage which might be chemical in origin. The effect does not appear in other traits thought to be often caused by fresh mutation, such as epiloia, neurofibromatosis and retinoblastoma, where only slight and statistically insignificant parental age increases have been registered. Mongolism would, by the same test, appear to have an entirely different cause (see fig. 1).

The investigation of parental age is only one part of the problem. The history of parental exposure to X-rays and other kinds of radiation needs to be recorded; occupational risks and possible exposure to chemical mutagens from external sources could also be made the subject of enquiry.

Table 3. Mean parental ages in sporadic cases of diseases attributed to fresh mutation compared with control mean ages

Disease	Source[1]		Control mean*	Number of cases		Excess over control mean (years)			
						Father's mean age		Mother's mean age	
Chondrodystrophy	*Mørch*	[1942]	D	97	176	+5.4	+5.1	+3.5	+3.6
	Krooth	[1952]	E	16		+6.8		+5.7	
	Grebe	[1955]	G	63		+4.3		+3.1	
Acrocephalosyndactyly	*Grebe*	[1944]	G	7		+5.5		+3.5	
Epiloia	*Gunther* and *Penrose*	[1935]	E	12	33	+0.8	+0.5	+0.3	+0.4
	Borberg	[1951]	D	21		+0.4		+0.5	
Neurofibromatosis	*Borberg*	[1951]	D	49		+0.9		+0.8	
Retinoblastoma	*Neel* and *Falls*	[1954]	M	64		+0.5		+0.7	
Mongolism	*Schulz*	[1931]	G	80	664	+5.3	+5.8	+7.7	+6.9
	Øster	[1953]	D	369		+5.3		+6.5	
	Penrose	[1955]	E	215		+6.8		+7.4	

[1] See *Penrose* [1956b]. * E England: father 30.9; mother 28.6, D Denmark: father 33.3; mother 28.6, G Germany: father 32.6; mother 28.9, M Michigan, U.S.A.: father 30.5; mother 26.4.

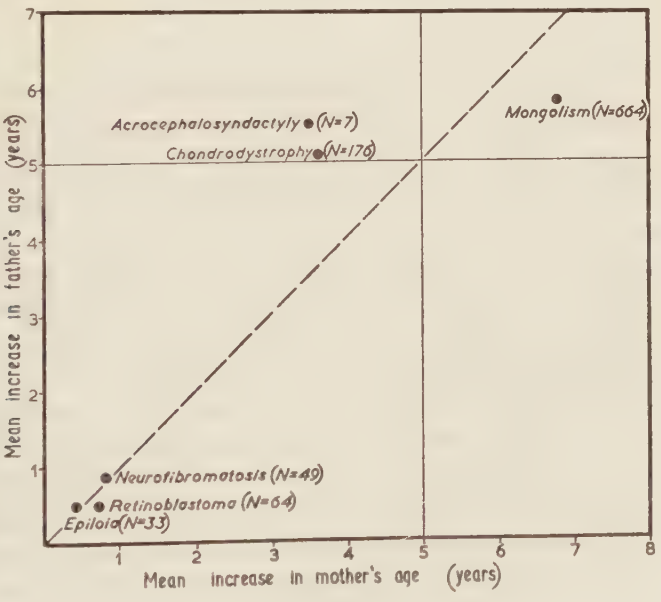

Fig. 1. Excess of parental ages above population averages in conditions thought to be due to fresh mutation.

(iii) *Observations on somatic cells*

It has been suggested that a tissue culture treated by exposure to a known dose of radiation could serve to investigate the sensitivity of human cells. Techniques for this purpose will no doubt be developed in time though such experiments may never be critical because germ cells could have different sensitivity from that of somatic cells. This objection may be for the moment left on one side, however, while we search for existing data which might give clues to mutation rate in somatic cells. The obvious source of information is observations concerning inductions of tumours by radiation.

It has until recently usually been assumed that very small amounts of ionizing radiation have no effect on the induction of leukaemia. This is now doubted and the relation between bone marrow dose and incidence of leukaemia is thought to be not unlike the linear effect observed in the induction of X-chromosome lethals in *Drosophila*. Some idea of the dosage to bone marrow required to double the spontaneous leukaemia rate can be obtained from published figures (*Court Brown* and *Doll* [1956]) and it is in the region of 30 to 50 röntgen units.

This line of thought leads to another interesting idea. The suggestion has been made that many sporadic cases of retinoblastoma arise as phenocopies. Is it not possible that these phenocopies are simply somatic mutations of the same kind as that which sometimes is carried in the germ track causing a dominant type of inheritance?

6. The "load" of abnormal genes in man

Finally, I would like to mention one or two points about the total effect of mutation on man since this has been so much discussed recently. Consider the total number of zygotes formed in a generation. We have no idea how many fail to pass through the first few divisions and never develop into embryos. Indeed it is impossible to deduce how many embryos are lost in the first six weeks after fertilization. According to *Yerushalmy* [1945] 15 per cent of human pregnancies are known to terminate in miscarriages or abortions. Beyond this, 3 per cent are stillborn and 2 per cent are neonatal deaths. In addition, early mortality after the first month amounts to 3 per cent. These are figures for European and North American communities, where infectious diseases and malnutrition are under efficient control. In many parts of the world they would be gross underestimates. Among those who survive to adult status, 20 per cent are unmarried and of those who do marry some 10 per cent are

infertile. How much of this continuous loss of zygotes, which may amount to about 50 per cent, is genetic is not known; by analogy with results obtained on ordinary metrical traits such as stature and intelligence, about half of this loss of zygotes might be directly hereditary. Perhaps the main factors are recessive lethals. If this were so, the indirect argument would lead to the conclusion that about a quarter of zygotes are lost each generation and that the genes which are thereby eliminated are replaced by fresh mutations. This points to the further conclusion, that a large increase in mutation rate, say permanent doubling, would eventually increase this lethal load to a half and would greatly reduce human fitness, though the immediate effects would be small. However, for reasons given earlier, I do not suppose this picture to be an accurate one. Much of the permanent lethality which we experience is likely to be due to balanced genetical mechanisms which do not require the assumption of appreciable amounts of mutation to maintain them. As I have previously pointed out (*Penrose* [1955b]) improved living conditions are likely to reduce the frequencies of recessive genes whose prevalence is due to heterozygous advantage. Thus genetic damage which may be done by increase of mutation rate, due to industrial and medical uses of radiation, may be offset in the future by the improvements in hygiene which are taking place at the present time all over the world.

REFERENCES

Böök, J.A.: A genetic and neuropsychiatric investigation of a North-Swedish population with special regard to schizophrenia and mental deficiency. Acta genet. *4*, 1, 1953.

Court Brown, W.M. and *R. Doll:* Leukaemia and aplastic anaemia in patients treated with X-rays for ankylosing spondylitis. Hazards to man of nuclear and allied radiations, Appendix B., London. H.M.S.O. 1956.

Crow, J.F.: A comparison of fetal and infant death rates in the progeny of radiologists and pathologists. Amer. J. Roentgenol. *73*, 467, 1955.

Fisher, R.A.: The genetical theory of natural selection. Clarendon Press, Oxford 1930.

Goodman, H.O. and *S.C. Reed:* Heredity of fibrosis of the pancreas, possible mutation rate of the gene. Amer. J. hum. Genet. *4*, 59, 1952.

Grebe, H.: Chondrodysplasie. Ed. dell'Istituto Gregorio Mendel, Roma 1955.

Haldane, J.B.S.: The causes of evolution. Longmans Green & Co., London 1932.

— The spread of harmful autosomal recessive genes in human populations. Ann. Eugen., London, *9*, 232, 1939.

— Mutation rate of the gene for haemophilia and its segregation ratios in males and females. Ann. Eugen., London, *13*, 262, 1946.

— Mutation in the sex-linked recessive type of muscular dystrophy. A possible sex difference. Ann. hum. Genet., London, *20*, 344, 1955.

Levine, P., E. Robinson, M. Celano, O. Briggs and *L. Falkinburg:* Gene interaction resulting in suppression of blood group substance B. Blood *10*, 1100, 1955.

Macht, S.H. and *P.S. Lawrence:* National survey of congenital malformations resulting from exposure to roentgen radiation. Amer. J. Roentgenol. *73*, 442, 1955.

Neel, J.V.: The population genetics of two inherited blood dyscrasias in man. Cold Sp. Harb. Symp. quant. Biol. *15*, 141, 1951.

Neel, J.V. and *W.J. Schull:* Human heredity. University of Chigago Press, Chigago 1954.

Penrose, L.S.: Parental age and mutation. Lancet *2*, 312, 1955a.

— Genetics and medicine. Advanc. Sci., London, *11*, 387, 1955b.

— The spontaneous mutation rate in man. Hazards to man of nuclear and allied radiations, Appendices C, D and E. H.M.S.O., London 1956a.

— The mutational origin of hereditary eye diseases. Mod. Probl. Ophthal. *1*, 501–511. S. Karger, Basel/New York 1956b.

Sjögren, T.: Die juvenile amaurotische Idiotie. Berlingska Boktryckeriet, Lund 1931.

Vanderpitte, J.M., W.W. Zuelzer, J.V. Neel and *J. Colaert:* Evidence concerning the inadequacy of mutation as an explanation of the frequency of the sickle cell gene in the Belgian Congo. Blood *10*, 341, 1955.

Vogel, F.: Über Genetik und Mutationsrate des Retinoblastoms (Glioma retinae). Z. Konst.lehre *32*, 308, 1954.

Yerushalmy, J.: Infant and maternal mortality in the modern world. Ann. Amer. Acad. Polit. Soc. Sci. *237*, 134, 1945.

Neel, J. V. and *W. J. Schull*: Acta genet. *6*, 183–196, 1956

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STUDIES ON THE POTENTIAL GENETIC EFFECTS OF THE ATOMIC BOMBS

By J. V. NEEL and W. J. SCHULL

The study to be described was undertaken as one facet of a comprehensive attempt to obtain information concerning the various possible delayed biological effects of exposure to an atomic bombing. So well known are the genetic effects of irradiation that inevitably one of the foremost questions in the minds of those considering the possible late consequences had to do with the characteristics of the children of exposed parents. We shall report on certain efforts made during the year 1946–1955 to answer the following two questions:

1. Can there be observed, during the first year of life, any differences between the children born to parents, one or both of whom were exposed to the effects of the atomic bombings of Hiroshima and Nagasaki, and the children born to suitable control parents, and
2. If differences do exist, how are these to be interpreted?

Data pertinent to these two questions were collected through the agency of the Atomic Bomb Casualty Commission (ABCC) of the National Academy of Sciences—National Research Council of the United States and with the assistance of the National Institute of Health of Japan. The study has involved the efforts of many, many people, to all of whom grateful acknowledgment is made in the detailed presentation now in press.

The possible observable genetic effects of irradiation upon the first generation born after an atomic bombing are many and varied. These include changes in the sex ratio, the frequency of stillbirths, the frequency of congenital malformation, infant mortality, etc. *Each of these indicators of genetic damage is also influenced by a number of other factors; there are*

no known unique yardsticks of genetic damage. Under these circumstances, the crux of any program of study was the feasibility of establishing control material which insofar as possible differed from the irradiated only with respect to the radiation factor. The kinds and quantity of data to be collected were shaped by a number of considerations, practical as well as scientific. Notable among these were the expected "smallness" of the radiation effects, and the expected "largeness" of non-radiation sources of variation.

Brief Description of the Program

Briefly, the plan of attack on the problem was as follows: In the post-war years there existed in Japan a ration system such that pregnant women upon registration of their pregnancy following the completion of the fifth month of gestation could acquire access to certain rationed items. With the cooperation of the city administrators of Hiroshima and Nagasaki, a system was instituted in 1948 whereby at the time of her registration for ration purposes, each pregnant woman or her representative in these two cities also registered with the ABCC and completed the first two-thirds of a questionnaire which included such items as identifying information, a brief radiation history of the husband and wife, a short summary of the past reproductive performance, and pertinent details concerning the present pregnancy. At the time of the termination of the pregnancy, the midwife or physician in attendance notified the Commission and completed the aforementioned questionnaire by answering certain questions pertaining to the characteristics of the child and delivery. More specifically, information was requested on the following possible indicators of a genetic difference between the children of control and irradiated parents: sex, birthweight, stillbirth, and presence of malformation. Fig. 1 is an English translation of the questionnaire.

Fig. 1. English Translation from the Japanese Investigation Sheet for Births after Atom Bomb. Printing of July, 1949.

1. Name of city and investigation sheet number		□□□□□□
2. Day, month, and year of registration		
3. Expected date of birth		□
	Husband	*Wife*
4. Name (Maiden name in case of wife)		
5. Birth dates of husband and wife		
6. Age (exact number of years and months)		□□ □□

7. Present in Hiroshima or Nagasaki at time of bombing
8. Location at time of bombing (street and number) □
9. Distance from hypocenter □□ □□
10. Indoors □
11. Type of building □
12. Did you have or not have subcutaneous bleeding □
13. Did you have or not have gingivitis □
14. Did you have or not have bloody diarrhea □
15. Did you have or not have epilation □
16. Did you have or not have fever □
17. Did you have or not have burns □
18. Did you have or not have external injuries □
19. Date, month, and year of beginning cohabitation
20. Number of months interruption of cohabitation
21. Total number of months cohabitation □□□
22. Number of months cohabitation before August, 1945 □□□
23. Number of pregnancies before August, 1945 □□
24. Number of spontaneous stillbirths before August, 1945 □
25. Number of therapeutic abortions before August, 1945 □
26. Number of months cohabitation after August, 1945 □□□
27. Number of pregnancies after August, 1945 (including present) □□
28. Number of spontaneous stillbirths after August, 1945 □
29. Number of therapeutic abortions after August, 1945 □
30. Total number of pregnancies □□
31. Total number of spontaneous stillbirths □
32. Total number of therapeutic abortions □
33. Marriage of blood relations (first cousin, one and one-half cousins, second cousins, etc.) □
34. Present address and occupation of husband
35. Day, month, and year of beginning of last menstrual period of wife
36. Day, month, and year of birth expectation (according to calculation sheet)
37. Present month of pregnancy
38. Day, month, and year of termination of birth □□

39. Number of weeks of pregnancy			□□
40. Course of labor:	Spontaneous	Induced	
	Duration	Use of instruments	□□
41. Condition of newborn	Live birth after 38 or more weeks	Premature birth under and including 38th week	
	Miscarriage 20 weeks or under	Stillbirth 21–29 weeks	
	Stillbirth 30–38 weeks	Stillbirth after 38 weeks	□
42. Multiple birth (2, 3, etc.)		Order of birth	□
43. Sex of newborn		44. Weight (grms)	□ □□□
45. Presence or absence of malformation			□
46. Type of malformation (give details)			
			□□□□□□
47. Date of death of newborn child			□□
48. Date of termination of any pregnancies after January, 1948			□
49. Remarks			
50. Name and address of attendant at birth			

Regardless of the type of termination, a Japanese physician in the employ of the Commission or the Japanese National Institutes of Health called to examine the child—at once, if there was a report of an abnormal termination or on a somewhat more leisurely schedule if the termination was reported as normal. The completeness of this system of reporting and follow-up was checked periodically by contrasting the number of births reported to the Commission with the number of births reported to municipal authorities. These studies indicated that approximately 93 per cent of births occurring in Hiroshima, and a somewhat higher percentage in Nagasaki, were known to the Commission. A large proportion of the 7 per cent not ascertained through the registration scheme subsequently came to our attention through other channels. These latter births, which we have termed the unregistered series, are not included in the results to be reported. They have been of value, however, in determining the magnitude of any bias introduced by the failure of the registration program to be exhaustive.

In the event that a pregnancy terminated abnormally, as in a stillbirth or a child with a congenital malformation, a supplementary questionnaire was completed in the patient's home by a physician in the employ of the ABCC. This questionnaire covered in some detail gynecologic

history, maternal illness during pregnancy, past reproductive performance, and economic status. In addition to this questionnaire, blood was obtained from the mother for a serological test for syphilis. This same supplemental questionnaire was routinely completed on every registration for which the terminal digit in the registration number was zero, that is to say, for every tenth registration.

The possibility had to be recognized that for a variety of reasons some malformations would not be diagnosed at birth. Accordingly, in 1950, a program was inaugurated to bring into the central clinical facility at age nine months as many of the children examined shortly after birth as possible. This afforded an opportunity to check on diagnostic oversights, to make supplementary diagnoses, and to collect more information on infant mortality. In addition, certain anthropometric measurements (height, weight, head and chest circumference) were obtained as an index of general physical development. Clinical facilities did not permit a 9-month follow-up on every registered termination, hence it was necessary to sample the terminations. This was accomplished by the simple expedient of calling in babies for examination according to the terminal registration digit of the pregnancy. Where a child who was included in the sample could not be examined, an attempt was made to establish why, in an effort to detect possible sources of bias. Other evidence pertinent to the question of irradiation effects was obtained from a study of early pregnancy terminations (those pregnancies terminating before the pregnant woman was eligible to register), and from the autopsying of as many as possible of the stillborn infants and those infants dying during the first few days of life.

The Evaluation of Parental Radiation Exposure

To analyze the data it was necessary to classify each pregnancy termination with respect to the exposure of the two parents. Five categories of exposure were recognized for each parent; hence a given pregnancy termination could be scored in one and only one of twenty-five exposure cells, the appropriate cell being determined by the conjoint parental exposure. The five exposure classifications are as follows:

1. Not present in Hiroshima or Nagasaki at the time of the bombing.
2. Present in one or the other of the two cities but at a distance from ground zero (a) greater than 3000 meters, or (b) 0–3000 meters and heavily shielded, or (c) 1500–3000 meters and moderately shielded, or (d) 2000–3000 meters and lightly shielded.

3. Present at a distance of (a) 2000–3000 meters and unshielded, or (b) 1000–2000 meters and lightly shielded, or (c) 0–1000 meters and moderately shielded.

4. Present but at a distance (a) less than 2000 meters and unshielded, or (b) less than 1000 meters and lightly shielded.

5. Present but less than 3000 meters from ground zero and exhibiting one or more of the following three symptoms of radiation sickness: epilation, petechiae, gingivitis.

"Heavy" shielding denotes presence in concrete or brick building or air raid shelter at the time of the bombing. "Moderate" shielding includes being within a street car, train, or car, behind a wall or under the eaves of a house on the side away from the hypocenter. Finally, "light" shielding includes those individuals giving their location as in a Japanese-style building or in a trench or behind a post or tree. From a consideration of what has been published concerning the distance-dosage curves of a "nominal" atomic bomb, the degree of shielding afforded by the structures enumerated above, and the levels of irradiation necessary to induce radiation sickness and/or leucopenia, it is estimated that these five categories of exposure correspond to doses of approximately 0, 5–10, 50–100, 100–150, and 200–300 roentgens equivalent physical respectively. The distribution of registered births by parental exposure is given in Table 1. Because of the relatively few individuals falling in Categories 4 and 5, these categories were combined for purposes of analysis.

Table 1a. Distribution of births by parental exposure (all births)

		HIROSHIMA					
		Mother's Exposure					
		1	2	3	4	5	Total
Father's Exposure	1	20192	6089	2499	462	855	30097
	2	1726	2145	488	100	142	4601
	3	697	452	594	54	75	1872
	4	155	127	93	34	27	436
	5	290	156	87	21	63	617
Total		23060	8969	3761	671	1162	37623

Table 1b. Distribution of births by parental exposure (all births).

NAGASAKI

Father's Exposure	Mother's Exposure 1	2	3	4	5	Total
1	16721	10398	851	121	492	28583
2	2497	4654	314	39	97	7601
3	269	309	118	14	22	732
4	50	56	14	6	1	127
5	111	138	26	2	22	299
Total	19648	15555	1323	182	634	37342

Extraneous Sources of Variation

As has been stated, all of the possible indicators of genetic damage utilized in this study are influenced by a variety of factors other than exposure. It was necessary, therefore, to undertake a detailed comparison of the parents of the infants comprising the various exposure subclasses with respect to certain possible differences which might influence the outcome of pregnancy. Time does not permit more than the briefest sketch of the result of this comparison. Consanguinity, maternal age, parity, economic status, frequency of positive serological test for syphilis, frequency of induced abortions and dilatation and curettage of the uterus, and the frequency of repeat registrations were among the factors studied. Of these, significant differences could be shown to exist among exposure subclasses with respect to the frequency of consanguineous marriages (the rate tended to decrease with increasing parental exposure), maternal age (the mean age tended to increase with increasing parental exposure as did the variance), and parity (mean and variance increase with increasing exposure). Each of these three factors exerts a rather appreciable effect on pregnancy outcome, with any of them quite probably equaling if not exceeding as a source of variation the expected effect of irradiation. The observed differences in maternal age and parity are such as to lead to higher frequencies of malformation, stillbirths, and neonatal deaths in the cells corresponding to the parents more heavily exposed even in the absence of an effect of exposure. Hence maternal age and parity differences could in these data lead to spurious irradiation effects. On the other hand, the observed differences in the frequency of consanguineous marriages are such as to lead to an increase in indicator values in the cells corresponding to the unexposed or lightly exposed parents and hence to a possible obscuring of irradiation differences. Among the other non-radia-

tion sources of variation only year of birth requires special comment. The bettering of the economic situation in Japan in the post-war years can be shown to be inversely correlated with the frequency of births to heavily exposed parents. To the extent that the bettering of the economic situation would be reflected in improved nutrition, year of birth can be shown to be of importance in determining the effect of parental exposure on birthweight.

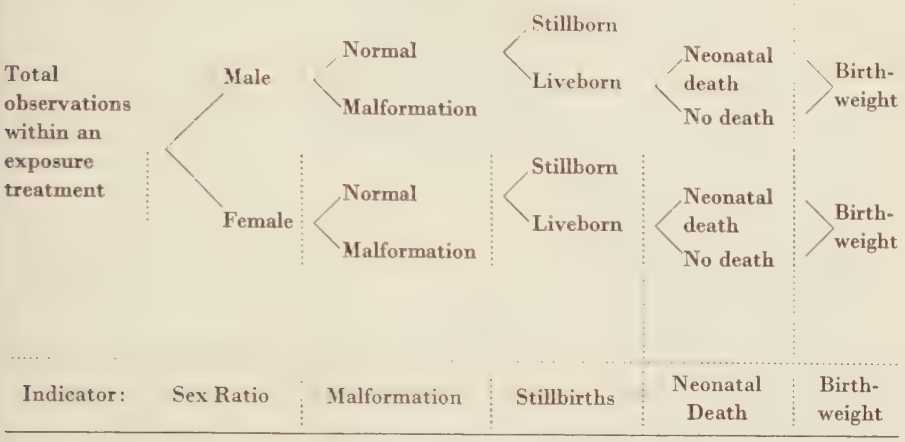
In the main the differences between exposure cells with respect to these non-radiation sources of variation are most pronounced between, on the one hand, the cells in which neither or only one of the parents were exposed and, on the other hand, the cells wherein both parents were exposed. Thus limiting one's attention to those cells where both parents were exposed minimized non-radiation sources of variation, and yet affords a good measure of the effect of irradiation since mean dose will vary from approximately 10 to 400–500 roentgens equivalent physical.

Statistical Methods

In view of the multiple problems which arise when one attempts to employ survey data in an analytical fashion, it is doubtful whether, given a body of survey data, any two competent statisticians would evolve essentially the same approach. While the basic question to be asked of the data is a relatively simple and straightforward one, namely, is there a difference between the outcome of pregnancy in irradiated and non-irradiated parents, the attempts to answer the question are complicated by three factors: (1) the possibly overlapping nature of some of the indicators, (2) extraneous (concomitant) variation, and (3) disproportionate numbers of observations in the various exposure cells. The first of these factors was readily met by a pyramidal handling of the data. Under the scheme employed, the first attribute to be handled was the sex ratio. This was followed by the frequency of malformation. In this and all subsequent partitions, sex was taken into account. All grossly malformed infants were then excluded, and the frequency of stillbirths obtained. The stillborn infants were discarded in turn, and birthweights distributed on the remainder. The order of the testing is indicated in Fig. 2.

But while it was relatively easy to handle the data so as to minimize the problem of overlapping indicators, making allowance for concomitant variation and disproportionate cell numbers was more difficult. Time does not permit even a sketchy presentation of the statistical techniques. Of the various types of concomitant variation enumerated above, three were

Fig. 2



felt to introduce potential biases of such magnitude that some effort at statistical adjustment was necessary. These were consanguinity, maternal age, and parity. Variation as regards the amount of consanguineous marriage among the parents of the children falling into the 25 radiation subgroups was met by the simple expedient of eliminating all children of consanguineous marriages from consideration. Variation in maternal age and parity was handled by a covariance analysis or by increasing the ways of classification, depending upon whether the variable was continously or discontinously distributed. In the latter case, information from the different ways of classification was pooled only after such pooling could be shown to be justified by the absence of significant interaction among the ways of classification. The techniques employed in testing for interactions will be presented in detail in the full publication now in press. The numbers of observations available in testing the various indicators are presented in Table 2, a and b.

With respect to the third of the analytic complications mentioned earlier, namely, disproportionate numbers of observations and the consequent non-orthogonality of the contrasts, in an analysis of variance we have relied primarily on the method of "fitting constants" described by *Wilks* [1938], with, in the case of the anthropometric studies on children aged 9 months, logical extensions of this method appropriate to the multi-variate analysis of dispersion.

This very brief description of the statistical methods, while mathematically entirely inadequate, may have at least served to indicate the main lines along which the analysis proceeded and the extremely labori-

Table 2a. An accounting of the number of observations considered at representative stages in the analysis of the "at birth" data and the number of rejected observations with the cause of rejection.

	Available Observations			Rejected Observations		
	Hiroshima	Nagasaki	Total	Hiroshima	Nagasaki	Total
Total infants seen	38421	38205	76626			
Rejected because the pregnancy was unregistered, parental exposure was unspecifiable, consanguinity or other observations were incomplete				3478	1868	5346
Considered for consanguinity	34943	36337	71280			
Rejected consanguinity				2113	2920	5033
Considered for maternal age	32830	33417	66247			
Rejected multiple births				365	451	816[1]
Considered for sex ratio	32465	32966	65431			
Considered for malformations	32465	32966	65431			
Rejected malformations				313	281	594
Rejected congenital heart disease				44	53	97
Total				357	334	691
Considered for stillbirths	32108	32632	64740			
Rejected stillbirths				472	482	954
Considered for neonatal deaths	31636	32150	63786			
Rejected neonatal deaths				414	480	894
Considered for birthweight	31222	31670	62892			

[1] In Hiroshima one set of registered triplets and 181 sets of registered twins occurred; in Nagasaki there was one set of registered triplets and 224 sets of registered twins.

ous computations involved. In closing this section we would like to express our particular appreciation for statistical help from *C. R. Rao* and *H. L. Lucas.*

Results

The results of the study are summarized in Table 3. For most of the indicators, two separate analyses have been presented, one including (the 4×4 case) and one excluding (the 3×3 case) those exposure cells wherein one or both parents were unexposed. The latter was deemed necessary because of the criticisms which can be leveled at use of the unexposed parents as controls. The comparisons given in this table are, in several instances, without correction for age-parity differences between exposure subclasses, a point we will refer to from time to time.

Table 2b. An accounting of the number of observations considered at representative stages in the analysis of the "9-months" data, and the number of rejected observations with the cause of rejection.

	Available Observations			Rejected Observations		
	Hiroshima	Nagasaki	Total	Hiroshima	Nagasaki	Total
Total infants on whom there exists some follow-up study .	14768	12324	27092			
Rejected inadequate exposure history, infant not part of 9-months program, etc.				3422	1882	5304
Total infants considered under the 9-months program . . .	11346	10442	21788			
Rejected consanguinity . . .				694	828	1522
Rejected incomplete measurements				140	308	448
Considered for neonatal death .	10512	9306	19818			
Rejected neonatal deaths . .				484	458	942
Considered for malformation . .	10028	8848	18876			
Rejected malformations . .				183	195	378
Considered for anthropometrics	9845	8653	18498			

Table 3a. A summarization of the comparisons of the various indicators with parental exposure when (a) all exposure cells are considered (the 4×4 case), and (b) only those cells where both parents were exposed are considered (the 3×3 case).

Indicator	Parental Exposure			
	Fathers		Mothers	
	4 × 4 case	3 × 3 case	4 × 4 case	3 × 3 case
Sex Ratio	.30–.50 ↑	.90–.95 ↑	.10–.20 ↓	.95–.98 ↓
Malformation: at birth	.50–.70 ↑	.80–.90 ↓	.50–.70 ↑	0.99 ↑
at 9 months .		.30–.50 ↓		.02–.05 ↓
Stillbirth	.20–.30 ↑	.80–.90 ↑	.001–.01 ↑	.30–.50 ↓
"Neonatal" Death	*	.20–.30 ↓	*	.02–.05 ↓
Death in 9 months		.95–.98 ↑		.50–.70 ↓
Birthweight Means:				
males-Hiroshima	.10–.25 ↓		>.25 ↓	
females-Hiroshima	>.25 ↓		>.25 ↓	
males-Nagasaki	>.25 ↑		.10–.25 ↓	
females-Nagasaki	>.25 ↑		.10–.25 ↑	
Anthropometrics:				
generalized means	<.001	.25–.50	.02–.05	.05–.10

* No general test.

Table 3b. A summarization of the comparisons of the various indicators with parental exposure when (a) all exposure cells are considered (the 4×4 case), and (b) only those cells where both parents were exposed are considered (the 3×3 case).

Indicator	Combined Parental Exposure	
	4×4 case	3×3 case
Birthweight Variances:		
males-Hiroshima	.10–.25	
females-Hiroshima	<.001	
males-Nagasaki	.10–.25	
females-Nagasaki	.10–.25	
Anthropometrics generalized variances:		
males-Hiroshima	.10–.25	>.25
females-Hiroshima	.10–.25	>.25
males-Nagasaki	.10–.25	>.25
females-Nagasaki	.05–.10	.05–.10

The figures in the columns refer to probability levels. The arrows indicate the direction of the difference between irradiated and control values. The arrow is directed upwards if there exists a continuing increase in the attribute or measurement under consideration as mean exposure increases, or downwards if the converse obtains. In the event the attribute or measurement bobbles, as it were, with increasing exposure, the direction of the arrow was determined by pooling exposure classes until a decision could be reached. The observations were weighted by the mean exposure of the class from which they were drawn. It will be at once apparent that most of the analyses have failed to reveal apparently significant relationships between indicator and parental radiation history. There are, however, a few specific points that merit discussion. In the order in which they appear in the table, the first finding at the level of significance is with respect to the frequency of malformation at age 9 months in relation to maternal exposure, in the 3×3 case. The downwards-directed arrow indicates a decrease in the frequency of malformation among the children of the more heavily irradiated, a finding which under most hypotheses would not be taken as evidence for increased mutation production. The second significant finding is an apparent increase in stillbirth frequency among the children of the more heavily irradiated mothers for the 4×4 case, but not for the 3×3 case. When, however, age and parity corrections are introduced, the apparent maternal exposure effect disappears for the 4×4 case. The third finding at the level of significance is neonatal

death rate in relation to maternal exposure for the 3×3 case. Significance here stems largely from a rather striking depression of the death rate among the infants born to mothers in Exposure Class 3. There is, however, a slight increase in the death rate in Exposure Class 4-5. The fourth and fifth findings concern the generalized means of the anthropometric examinations conducted at age 9 months, in relation to both maternal and paternal exposure for the 4×4 case. The disappearance of these apparent effects for the 3×3 case raises questions concerning their validity. The sixth and final significant findings concerns birthweight variances among female infants born in Nagasaki, with parental exposure considered jointly for the 4×4 case. This effect is not borne out by the three other comparable analyses of birthweight variances.

In summary, then, there emerge from this analysis no really clear indications that the radiation history of the parents has affected the characteristics of their children here under consideration. It should in this connection be pointed out that 5 of the 6 findings which give some indications of significance involve the element of maternal exposure, a fact which in view of the possibility of maternal somatic effects suggest the need for particular caution in reaching conclusions. In order to avoid all possible misunderstandings we hasten to state that under no circumstances can this study be interpreted as indicating that there were no genetic consequences of the atomic bombings. The interpretation is simply that *conclusive* effects could not be demonstrated.

In a preliminary communication concerning this study (*Neel* et al. [1953]), it was reported that there appeared to be a significant relationship between sex and parental exposure history, but no other positive findings. This relationship does not appear in the present analysis, although the direction of deviation is still the same. Among possible reasons for the disparity the following should be considered: (1) the different (improved) classification of parental exposure employed in the present analysis, and (2) the accumulation of additional data.

Thus far in our analysis we have been concerned with attempts to demonstrate a positive effect of exposure to the atomic bombs on the indicators selected for study. There is, however, another aspect of these data. They permit us to place upper limits on the effects which may have been induced but not demonstrated by these studies. In other words, we can place confidence limits on our observations. The approach employed has been to compute the power functions for our several tests, having first taken several steps to simplify the statistical computations. The most important of these steps involves limiting the computation to those

exposure cells with father's and mother's class 1 or 2 vs. those cells with father's and mother's class 3, 4, or 5. This step, by ignoring a portion of the data, has the effect of making our approach appear statistically less powerful than it really is. At any rate, on the basis of these computations, it can be stated that our data are adequate to give assurance at the 90 per cent level that we would be able to detect the following:

(i) a decrease in the sex ratio, following maternal exposure, in excess of an absolute change of 1.6 per cent;

(ii) an increase in the sex ratio, following paternal exposure, in excess of an absolute change of 4 per cent;

(iii) an alteration of the malformation rate in excess of two times the control value; and

(iv) an alteration of the stillbirth and neonatal death rates in excess of approximately 1.8 times the control value.

In concluding, we should like to be the first to recognize the unsatisfactory situation in which this study leaves us, with respect to drawing firm conclusions concerning radiation-induced genetic changes in human populations. There seems to be agreement at all levels that with the advent of the atomic age, one of the most pressing questions in the entire field of human biology, including medicine, concerns the genetic problems created by the exposure, for various reasons, of the human race to increasing amounts of high energy irradiation. The complexity of this problem is self-evident. The final evaluation on which a valid course of action can be based will depend on studies of many types, all carefully evaluated and cross-correlated. This study will have achieved its objective if in the ultimate synthesis it supplies one of the sets of observations to be taken into consideration.

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RADIATION GENETICS AND HUMAN POPULATIONS

By T. C. CARTER

Introduction

Eleven years ago the public at large became aware that ionizing radiations are always potentially dangerous to the human individual and may be lethal. More recently this has been followed, thanks largely to the warnings of *J. B. S. Haldane*, *H. J. Muller*, *A. H. Sturtevant* and *C. H. Waddington*, by the realisation that there is another and possibly more insidious hazard, namely that to the genetic material. This led to the appointment of national committees in Great Britain and the United States charged with reviewing existing knowledge of the long-term hazard of ionizing radiation, drawing such inferences as were possible and recommending how the hazard might be minimised.

These committees have now presented their reports. They differ in matters of detail, but in their main conclusion they are undivided; in particular, they point out to the governments concerned what has for long been obvious to any geneticist, namely that there are enormous gaps in our knowledge of radiation genetics, and especially of the radiation genetics of man, and that there is a pressing need for research to fill these gaps. The reports have been accepted. The prospect is therefore that support for genetical research may in the future become rather less difficult to obtain than has generally been the case in the past. The present is therefore a time when we should be taking stock and reviewing not only the details but also the fundamental concepts of our science, for geneticists may shortly be asked to define how additional support, if it were forthcoming, could most profitably be applied.

Radiation genetics is a duplex science, its two constituent elements being concerned with, respectively, the induction of mutants in populations and their subsequent elimination. In the last thirty-five years much research has been concerned with the induction of mutation, not only by radiations but also by chemical mutagens. Much less effort has been spent on studying how mutants are eliminated. It is primarily this aspect that I wish to consider in the present paper.

Mathematical Models of Populations

The relative rarity of population studies is undoubtedly due largely to the great technical and statistical difficulties inherent in such work and its interpretation. However, it may also be due, at least in part, to the existence of a mathematical population model, developed by *J. B. S. Haldane*, *Sewall Wright*, *R. A. Fisher* and others, which is aesthetically so satisfying that the arduous study of actual populations, as they exist in nature, may at times have appeared almost unnecessary. Furthermore, geneticists have not always made a clear distinction between the mathematical abstraction and the biological reality. I now want to draw attention to two points. First, that most published calculations of the genetic hazard to man are based on a simple form of the mathematical model in which it is assumed that all mutation must be balanced by selective elimination; and, second, that this assumption may cease to be valid if a slightly more complicated model is used, incorporating some degree of gene interaction within and between loci and of environmental fluctuation, all of which are known to operate in natural populations.

The simple model of a sexually reproducing diploid population consists of an indefinitely large number of individuals, each equipped with a large number of pairs of genes. The genes of each pair can exist in either of two allelic forms, wild-type and mutant. Each form mutates to the other with a low but definite probability which can be enhanced by mutagenic treatment. Alleles segregate one-to-one in heterozygotes; gene pairs segregate independently. A zygote carrying a mutant allele has a lower biological fitness than its wild-type counterpart, contributing fewer progeny, on the average, to the next generation. The effect on fitness of substitution in one pair of genes is independent of that in other pairs. The population is panmictic and inhabits an unchanging environment.

The Balance of Mutation and Selection

The first inference drawn from the simple model is that an autosomal dominant mutant allele which is unconditionally harmful, invariably

reducing the biological fitness of its carriers, will be maintained in the population only by recurrent mutation and at a low frequency. A state of genetic equilibrium will be set up, at which the increase of mutant alleles through mutation from the common wild-type allele is balanced mainly by loss through natural selection. There will be a little loss through back mutation, but it will be slight because of the low frequency of mutant alleles; it is usually neglected. There can be little doubt that this is a reasonably accurate picture of the situation in respect of many seriously harmful conditions in human populations, such as epiloia and retinoblastoma, to name but two. In such instances the existence of the mutant allele is the cause of much suffering by the afflicted individuals and their close relatives, and it imposes a social load on the whole population through the necessity to provide medical care. These, and these alone, are sufficient reasons for us to deplore any unnecessary general increase in mutation brought about by unnecessary exposure to ionizing radiations or other mutagens.

The concept of genetic equilibrium through the balance of new mutation with natural selection has, however, been applied far more widely than to grossly harmful autosomal dominants. It has been used to support the view that all mutation is harmful, or very nearly all. It has been argued that all mutable genes must have mutated at some time in the past, and therefore any advantageous allele which could arise must already have arisen; it would then have spread through the population under the action of natural selection, being incorporated into the current wild-type genome. Any mutation away from the wild-type would therefore be to a less advantageous allele. To maintain genetic equilibrium, every mutant allele arising by mutation from the wild-type, no matter how slight its effect on fitness, would have to be eliminated from the population, sooner or later, through the early death or reduced fertility of some individual. The smaller the effect on fitness, the longer would each mutant allele persist in the population before elimination, and the greater the number of individuals it would affect. Thus there would be an inverse relationship between the amount of harm to an individual and the number of individuals harmed, so that all mutations would tend to cause the same integrated amount of harm to the population as a whole. The total mutation rate would then be a measure of the total genetic load.

The Balance of Opposed Selective Forces

The above argument rests on the assumption that all genes, or nearly all, exist as common, advantageous, wild-type alleles or rare,

deleterious, mutant alleles; and that all increase of mutant alleles through mutation must be balanced by loss through natural selection. My object now is to point out that there is a situation in which this argument breaks down, namely that in which both alleles have fairly high frequencies and the balance is not between mutation and natural selection but between positive and negative selective forces. It may occur whenever a given allele confers an advantage on some members of a population and a disadvantage on others. There are at least three important conditions under which it may arise: selective advantage of the heterozygote in a single-factor system, selective advantage of the central phenotype in a polygenic system, and temporal or spatial variation of the environment within the range of the population. Under all of these conditions the distinction between wild-type and mutant alleles may become blurred; they may coexist in the population, each with a high frequency; and mutation may play only a small part, or none at all, in determining the equilibrium frequency of the alleles in question.

(*i*) *Balanced polymorphism*

Theoretically the simplest and most extreme case is that of a balanced single-factor polymorphic system in which two alleles occur with equal frequencies and their forward and back mutation rates are equal. In this situation increase of the mutation rates, both equally, would have no effect whatsoever; any additional forward mutation would be exactly counterbalanced by back mutation and the net effect on the population would be nil. The net effect of mutation would still be nil with unequal forward and back mutation rates, if they were in inverse proportion to the allele frequencies. For other values of the mutation rates there would be some net effect, but it would be small so long as the rarer allele had a fairly high frequency. This type of system has long been recognised as a theoretical consequence of heterozygous advantage: that is to say, it will tend to occur wherever a heterozygote has a higher biological fitness than either of the corresponding homozygotes. *E. B. Ford* and his co-workers have shown that this type of system operates in many polymorphic populations of Lepidoptera; *Dobzhansky* and his school have shown that cryptic polymorphism in *Drosophila*, involving chromosomal arrangements, has a similar basis; and *L. C. Dunn* has shown that biological advantage in heterozygous males, here operating through non-mendelian segregation, underlies the polymorphism in respect of *t*-alleles found in most wild mouse populations. Heterozygous advantage is less certainly established as a cause of polymorphism in human populations, perhaps

because of the difficulty of measuring small selective forces in man. There is a tendency to assume that it is in operation whenever a human population shows polymorphism in respect of a single-gene character, but only in a few instances has evidence been brought forward to support the assumption. A notable exception is the sickle-cell polymorphic system among African negroes, where there is evidence that heterozygotes are unusually resistant to infection with malaria (*Allison* [1954]). Other exceptions relate to gastric carcinoma and peptic ulceration, on the one hand, and the ABO blood group system on the other; in four bodies of data the wholly heterozygous AB class showed a lower incidence of the disease than did the wholly homozygous O or the partly homozygous A and B classes (*Aird*, *Bentall* and *Fraser Roberts* [1953], *Holländer* [1953], *Buckwalter*, *Wohlwend*, *Colter* and *Tidrick* [1956]).

(*ii*) *Optimal central phenotype.*

The same essential feature is present in a polygenic system where the central phenotype is at a selective advantage; thus a minor gene tending to increase expression of the character is at an advantage in individuals with suboptimal expression and a disadvantage in supraoptimal individuals. In man many genes affecting intelligence must come into this category, since individuals with average intelligence are more fertile than both those with very low and those with very high intelligence, as *Penrose* [1955] has pointed out. Furthermore, intelligence is not the only human quantitative character in which the central expression is at a selective optimum.

(*iii*) *Environmental fluctuation*

A third theoretically possible cause of a situation in which two alleles coexist with high frequencies is environmental fluctuation. The selective value of any allele is defined in relation to the environment. An allele conferring only a slight selective disadvantage in one environment might therefore be carried across the threshold to selective advantage by only a small environmental change. It would then tend to spread, under the action of natural selection, until reversal of the environmental change rendered the allele disadvantageous once more and so reversed its spread. It seems reasonable to expect that such alleles may be common in nature, for on general grounds one might expect an inverse relationship between the degree of detriment due to an allele and the frequency of loci at which such alleles could arise. If this were so, loci with only slightly disadvantageous alleles might be relatively common and alleles held at a high frequency by environmental fluctuation might constitute quite a large class. The

extent to which such genes may occur in man is at present unknown; but one may ask whether this class includes some of the well-known alleles which are widespread in human populations but have only trivial effects on the phenotype and no obvious selective value. The genes responsible for minor metabolic or sensory anomalies come to mind as possible examples.

Implications for Human Genetic Research

The arguments given above lead to the conclusion that there are at least three sets of circumstances which may theoretically give rise to a situation in which two or more alleles are held at high frequencies by opposed selective forces; and that in such a situation the dysgenic effect of an increased mutation rate will be reduced or even eliminated entirely. There is a great mass of evidence that the human species is genetically very heterogeneous; and this suggests the possibility, to put it no higher, that such a situation may be of widespread occurrence in human populations. If so, serious overestimates of the genetic hazard may have been obtained from calculations based on the assumption that all human genes exist only as unconditionally advantageous or unconditionally deleterious alleles. However, the path from a general impression to a clear-cut demonstration or, alternatively, to a disproof is often a long one. Nevertheless, since the value of any such calculation depends entirely on the extent to which this assumption is valid, I think it is a path which human geneticists are bound to take.

Though different views may be taken of the proportion of human alleles which come into the unconditionally deleterious category, there can be no doubt that many do so. Furthermore, it is they that contribute a heavy part of the social load on our populations. This alone gives sufficient reason for avoiding any unnecessary radiation exposure, for in them the full effect of any change of mutation rate will be felt. The implications for human genetic research are therefore that we should concentrate on identifying, first, those parts of the social load which have a genetic basis and, second, the genetic mechanism underlying each clinical condition; this should include estimates of the extent to which environmental factors enter and, where single-gene segregations are identifiable, of the frequencies and spontaneous mutation rates of the alleles concerned and the biological fitness of affected individuals. Only when this has been done will it become possible to start on the next stage of the work, which is to calculate with any confidence the effect of an induced change in the mutation rate.

Summary

The outcome of calculations of the long-term genetic hazard to man from ionizing radiations depends on the assumptions made about the underlying genetic mechanisms. Most calculations have assumed that all mutant alleles are unconditionally detrimental and must be eliminated by natural selection. This may be invalid in at least three circumstances, namely (i) heterozygous advantage, (ii) optimum central phenotype and (iii) environmental fluctuation; more research is needed to find how far these obtain in human populations. The assumption of unconditional detriment is probably true of many of the mutant alleles responsible for the present social load of genetically conditioned illness in human populations and for this reason alone any unnecessary radiation exposure must be avoided. Estimation of the dysgenic effect of a mutation rate increase requires detailed knowledge of the genetic mechanism underlying the illnesses which constitute the social load; obtaining this knowledge is the first task facing human geneticists.

REFERENCES

Aird, I., H. H. Bentall and *J. A. Fraser Roberts:* A relationship between cancer of the stomach and the ABO blood groups. Brit. med. J. *1*, 799–801, 1953.

Allison, A. C.: Protection afforded by sickle-cell trait against subtertian malaria infection. Brit. med. J. *1*, 290–294, 1954.

Buckwalter, J. A., E. B. Wohlwend, D. C. Colter and *R. T. Tidrick:* Natural selection associated with the ABO blood group. Science *123*, 840–841, 1956.

Holländer: Unpublished data quoted by *Aird* et alii, 1953.

Penrose, L. S.: Evidence of heterosis in man. Proc. roy. Soc. (B) *144*, 203–213, 1955.

Turpin, R., J. Lejeune et *M.-O. Rethore:* Acta genet. *6*, 204–216, 1956

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ÉTUDE DE LA DESCENDANCE DE SUJETS TRAITÉS PAR RADIOTHÉRAPIE PELVIENNE[1]

Par R. TURPIN, J. LEJEUNE et M.-O. RETHORE

Introduction

Dans le but de mettre en évidence d'éventuelles mutations léthales liées au sexe induites chez l'homme par l'énergie ionisante nous avons étudié la descendance de sujets traités par R.X. sur la région pelvienne. Les cas de cancers ont été écartés de cette statistique en raison de leur mortalité élevée et de la stérilité quasi totale qui suit la radiothérapie, du fait des très fortes doses utilisées.

Notre étude a été essentiellement centrée sur l'évolution de la sex ratio avant et après traitement. Bien entendu les informations concernant les avortements, les mort-nés, les jumeaux et les malformations congénitales ont été relevées. Nous avons même noté un léger accroissement de ces phénomènes après traitement. Mais en raison de leur petit nombre, de leur déterminisme génétique souvent mal connu, et de l'influence sur leur apparition de l'âge maternel, nous n'avons pu les considérer comme probants. D'ailleurs, le premier temps de l'enquête ayant été réalisé par questionnaire, il persiste un doute sur le diagnostic des anomalies signalées, que seul l'examen systématique de toutes les familles recensées (deuxième temps actuellement en cours) permettra de lever.

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I. Présentation de l'enquête

A. Sélection des malades

Dans le but de recueillir le plus grand nombre possible de cas apportant une information, les fichiers de *tous les services de radiothérapie des hôpitaux parisiens et de la région parisienne* ont été examinés. Au total sur les 238.800 dossiers complets recensés dans 20 hôpitaux différents on a dénombré 4.428 cas d'irradiation pelvienne. Ces irradiations étaient justifiées par des affections variées, mais non cancéreuses, chez des adultes jeunes (femmes de moins de 35 ans et hommes de moins de 40 ans). Cette limite d'âge a été fixée dans le but de sélectionner des individus physiologiquement aptes à la procréation au moment du traitement. Tous les traitements relevés ont été effectués avant le mois de décembre 1952, les années recensées remontant jusqu'à 1940 pour la plupart des hôpitaux, jusqu'à 1930 et même 1925 pour certains.

Dans tous les cas retenus la dose de roentgens appliquée, la qualité du rayonnement (kilovoltage, milliampérage, distance ampoule-sujet et filtres), le champ d'irradiation, les dates de début et de fin de traitement et l'affection motivant ce dernier étaient spécifiés.

Nous noterons que sur ces 4.428 cas, 3.579 se rapportaient à des hommes, alors que l'on ne comptait que 849 femmes. Ceci tient sans doute à la fréquence plus ou moins grande des indications de la radiothérapie pelvienne suivant le sexe, mais peut-être aussi à la réserve des radiologistes à l'égard d'une thérapeutique dont les effets peuvent se traduire rapidement par l'aménorrhée.

Dans le premier temps de cette enquête un questionnaire permettant d'établir la descendance de l'individu a donc été envoyé à chacun des 4.428 sujets sélectionnés, et des rappels plusieurs fois répétés ont été adressés aux personnes qui n'avaient pas répondu à la 1e lettre.

Avant d'aborder l'étude des résultats enregistrés, il est important de signaler que les doses de roentgens qui seront mentionnées représentent toujours la quantité d'énergie ionisante délivrée à la peau d'après l'estimation même du radiothérapeute qui effectua le traitement. En aucun cas nous n'avons fait le calcul de la fraction du rayonnement ayant pu atteindre directement ou indirectement les gonades. Ce calcul est en effet pratiquement impossible, ou ses résultats sont tout au moins entachés d'une très grande incertitude, en raison des précisions relativement minimes que nous possédons sur la direction exacte du faisceau, aussi bien que sur les caractéristiques corporelles du sujet.

B. Dénombrement de la descendance des hommes irradiés

Sur les 3.579 questionnaires envoyés, 1.334 (soit 37,3 %) correctement remplis et utilisables pour notre propos, nous ont été retournés.

Ces 1.334 réponses concernent évidemment des sujets traités pour des raisons et dans des conditions différentes. Nous avons été amenés, en conséquence à les répartir en trois catégories:

a) 368 sujets irradiés sur la région lombaire haute ou sur la cuisse.

Pour la plupart (320) ces sujets atteints de névralgie sciatique ont été irradiés sur la région lombaire *haute*, les autres, 48, ont été irradiés sur la cuisse pour des motifs variés. Ce groupe est donc exclu de la présente statistique.

b) 180 sujets irradiés sur la région pelvienne, mais dont les gonades ont du être, en principe, protégées contre les radiations. En effet, lors des irradiations par voie antérieure, il est de règle de protéger les testicules par une lame de plomb reposant sur les cuisses. On ne peut malheureusement pas déterminer dans quelle mesure cette règle a été suivie.

Ces 180 sujets comprennent:

109 Rhumatisme ou arthrose coxo-fémorale

64 Adénopathies inguinales (*Nicolas Favre* surtout, ces sujets étant d'ailleurs presque tous stériles après traitement)

et 7 irradiations sur la région pubienne et sus-pubienne (dermatoses).

Parmi ces 180 individus probablement protégés, donc ayant reçu un taux faible de roentgens, on trouve 66 sujets ayant eu avant traitement:

Nés vivants			Mort-nés			
♂	♀	Avortements	♂	♀	Jumeaux	Tarés
62	50	12	3	0	0	2

et 52 sujets ayant eu après traitement.

Nés vivants			Mort-nés			
♂	♀	Avortements	♂	♀	Jumeaux	Tarés
42	54	13	4	1	0	0

soit, avant traitement, une sex ratio de 0,554 ± 0.050 et de 0,437 ± 0,051 après traitement. Cette sex ratio post radiothérapique assez particulière rend délicate l'élimination de cet échantillon du reste de la statistique[1].

[1] Nous noterons à ce propos que d'après nos premières constatations, il n'est pas exclu qu'une irradiation très faible des gonades entraîne chez l'homme une *diminution* de la sex ratio. L'étude de cette possibilité est actuellement en cours.

Cependant, comme ces fluctuations ne dépassent pas ce que l'on pourrait attendre du simple hasard, l'exclusion de ces données, motivée par les modalités mêmes de l'irradiation, paraît légitime.

c) 786 sujets irradiés sur la région pelvienne et dont les gonades n'ont pu être protégées des radiations. Ces sujets se répartissent en deux catégories:

1. 517 sujets tous traités par radiothérapie postérieure centrée sur l'interligne lombo-sacré et les deux premières pièces sacrées pour névralgie sciatique d'une part,

2. 269 sujets traités pour des motifs variés, soit par voie postérieure sur la région lombo-sacrée, soit par voie antérieure directement sur la région ano-génitale. Les différentes causes d'irradiation se répartissent ainsi:

120 traités directement sur les organes sexuels (prurit ou dermatose) le périnée ou l'anus (hémorroïdes)
59 douleurs sacro-iliaques
79 Rhumatisme lombo-sacré,
11 spina bifida lombo-sacrée.

Comme ces deux échantillons ne peuvent être considérés comme rigoureusement comparables, nous les détaillerons séparément.

Répartition des 786 cas d'irradiation pelvienne non protégés.

	Motifs divers		Sciatiques	
		age de la conjointe (au moment du traitement)		age de la conjointe (au moment du traitement.
Célibataires	38		52	
Mariés sans enfants	41	29,5 ans	79	28,6 ans
Ayant eu des enfants seulement avant . . .	95	31,6 ans	192	32,6 ans
Ayant eu des enfants seulement après . . .	53	23,5 ans	102	25,0 ans
Ayant eu des enfants avant et après	42	28,6 ans	92	28,3 ans
	269		517	

C. Dénombrement de la descendance de femmes irradiées

Sur les 849 femmes sollicitées, 284 soit 33,5% nous ont fourni des réponses utilisables.

Ces 284 observations se décomposent ainsi:

20 irradiation du périnée (prurit vulvaire, dermatoses, hémorroïdes)
58 névralgie sciatique
20 douleurs sacrées

14 arthrite sacro-iliaque
54 lésions coxo-fémorales
9 adénopathies inguinales
17 troubles ovariens (pour dysménorrhée)
18 région sus-pubienne (dermatoses)
2 spina bifida lombaire
43 région lombaire
et 29 motifs variés.

et se distribuent de la façon suivante:

		Age moyen au moment du traitement
Célibataires	44	
Mariées sans enfants	34	28,2 ans
ayant eu des enfants seulement avant . .	109	31,8 ans
ayant eu des enfants avant et après . . .	45	35,2 ans
ayant eu des enfants seulement après . .	52	35,2 ans
	284	

On trouvera dans le tableau suivant (Tableau I) la descendance, avant et après traitement des individus recensés.

Tableau 1. Descendance des sujets ayant répondu aux questionnaires.

		Total		Mort-nés		Av.	Tares		Jumeaux		
		M	F	M	F		M	F	MM	MF	FF
Avant traitement	Hommes (137)										
	Motifs divers	116	115	0	0	17	1	1	0	0	0
	Hommes (284)										
	Sciatique	242	223	4	3	29	3	3	3	0	3
	Femmes (154)										
	Total	130	106	2	3	18	1	0	0	1	2
Après traitement	Hommes (95)										
	Motifs divers (1.461 r).	68	62	2	2	20	2	2	3	0	1
	Hommes (194)										
	Sciatique (1.295 r) . .	157	118	3	1	27	2	3	1	1	2
	Femmes (97)								(triplet mâle)		
	Total (1.360 r) . . .	63	73	6	1	26	1	4	1	1	0

II. Etude de la sex ratio

De la structure chromosomique du sexe, il résulte que des mutations léthales éventuelles (les seules decelables par l'étude de la sex ratio) se manifesteront différemment dans la descendance des sujets traités selon le sexe du procréateur irradié.

En effet, dans la descendance d'une femme traitée, une mutation léthale dominante liée au chromosome X ne peut avoir d'effet sur la sex-ratio, tandis que les mutations récessives liées au sexe entraîneront un déficit des garçons donc une diminution de la sex-ratio. Au contraire chez les hommes, les mutations dominantes léthales liées au chromosome X pourront seules se manifester en entraînant un déficit de filles, donc une augmentation de la sex-ratio.

I. Sex ratio de la descendance des hommes irradiés

Pour les raisons qui viennent d'être exposées cette étude a été effectuée sur un échantillon de 786 sujets traités pour névralgie sciatique ou autres motifs, sans protection des gonades.

a) *les 92 individus (névralgie sciatique) ayant eu des enfants avant et après traitement* représentent évidemment un matériel idéal pour étudier les variations de la sex ratio avant et après le traitement. On trouve en effet:

	♂	♀		Total	Sex ratio
avant traitement	79	71	=	150	0,526±0,042
après traitement	66	53	=	119	0,555±0,045

b) de même pour les 42 pères (motifs variés) ayant eu des enfants avant et après traitement on trouve:

	♂	♀		Total	Sex ratio
avant traitement	30	37	=	67	0,448±0,060
après traitement	27	24	=	51	0,530±0,071

En combinant les deux échantillons nous avons donc 134 pères ayant eu avant et après traitement:

	♂	♀		Total	Sex ratio
avant traitement	109	108	=	217	0,502±0,035
après traitement	93	77	=	170	0,547±0,038

la différence des sex ratio n'est pas significative mais se trouve être dans le sens attendu de l'augmentation.

c) Si nous combinons alors la descendance de *tous* les sujets ayant eu des enfants avant traitement, pour la comparer à celle de *tous* les sujets ayant eu des enfants après, nous arrivons au tableau suivant:

		♂	♀		Total	Sex ratio
avant R.X.	Sciatique (284 pères)	242	223	=	465	0,520±0,024
	Tous motifs (137 pères)	116	115	=	231	0,502±0,034
après R.X.	Sciatique (194 pères ayant reçu en moyenne 1.295 r)	157	118	=	275	0,571±0,030
	Tous motifs (95 pères, 1.461 r) .	68	62	=	130	0,523±0,044

Soit, en combinant les deux échantillons une sex ratio de 0,514 ±0,019 sur 696 enfants nés avant traitement contre 0,555 ±0,026 sur 405 enfants nés après.

Ces différences, quoique toujours orientées dans le sens d'une augmentation de la sex ratio après traitement, ne sont pas non plus significatives.

b) Il est cependant intéressant d'examiner de quelle façon se produit cette augmentation de la sex ratio en fonction de l'accroissement des fratries après traitement. Dans l'hypothèse de la production de léthaux dominants, décelés seulement sur le chromosome X, la déviation devrait en effet être d'autant plus importante que l'individu est plus proche de la stérilité. Cette étude en fonction du développement de la taille des fratries après traitement n'est évidemment qu'une première approche du phénomène puisque l'on ne peut ainsi tenir compte, ni de la stérilité physiologique due à l'âge des conjoints, ni de la réduction volontaire des naissances.

Ces restrictions une fois posées, on observe la répartition suivante:

Taille des fratries après traitement

		1	2	3	4	5
Sciatiques (194 pères) . .	♂	64	59	18	11	5
	♀	45	40	17	11	5
Motifs divers (95 pères) . .	♂	23	21	8	13	3
	♀	27	22	8	5	0

Soit, en combinant les deux échantillons dont les variations sont, en gros, parallèles:

Taille des fratries après traitement

		1	2	3	4	5
Total (289 pères)	♂	87	80	26	24	8
	♀	72	62	25	16	5
		159	142	51	40	13

En accord avec les prévisions la variation de la sex ratio est maximum, pour les fratries de 1 et 2 enfants dans l'échantillon de sciatiques, mais le même phénomème est moins marqué pour l'échantillon général.

e) Par ailleurs, la répartition de la sex ratio d'après le temps écoulé entre la fin de l'irradiation et la date de naissance de l'enfant, conduit à la figure suivante:

Années écoulées[1]		0–1	2–3	4–5	6–7	8–9
Sciatiques	♂	23	49	41	24	20
	♀	24	34	22	21	17
Motifs variés	♂	10	18	14	8	18
	♀	13	17	10	10	12
Soit au total:						
Années		0–1	2–3	4–5	6–7	8–9
Sciatiques et Motifs variés	♂	33	67	55	32	38
	♀	37	51	32	31	29

* Le symbole 0 indique que l'enfant a été conçu alors que le père était en cours de traitement – cinq cas de ce type ont été relevés: 3 ♂, 2 ♀.

Ces variations, en apparence désordonnées, ont l'avantage de montrer que, s'il existe réellement une augmentation de la sex ratio due à la production de léthaux dominants liés à l'X, il n'y a aucune raison de penser qu'elle porte essentiellement sur les enfants nés immédiatement après le traitement. Autrement dit, ces données permettent de penser que si le déficit de filles est une réalité (que nos chiffres ne permettent d'ailleurs pas d'affirmer) *il doit se produire essentiellement sur des sujets issus de gamètes paternels irradiés au stade spermatogonial.*

f) Un dernier découpage des données permet d'étudier les variations de la sex ratio en fonction de la dose reçue.

Nous observons alors au total

Sciatiques et motifs divers	♂	♀	Sex ratio
Avant traitement			
432 pères	358	338 = 696	0,514±0,019
Après traitement			
Descendance de pères ayant reçu moins de 1.000 ($\bar{r} = 721$)	85	76 = 161	0,528±0,039
Descendance de pères ayant reçu plus de 1.000 ($\bar{r} = 1.730$)	140	104 = 244	0,574±0,032

Les enseignements que l'on peut tirer de cette étude de la sex ratio avant et après traitement dans la descendance d'hommes irradiés sur la région pelvienne sont les suivants:

1. Les déviations observées, quoique non significatives, se font dans le sens d'une augmentation de la sex ratio, ceci en plein accord avec l'hypothèse génétique d'induction de léthaux dominants liés à l'X;

2. La sex ratio est d'autant plus augmentée que l'accroissement de la fratrie après traitement est plus petit et, d'autre part, que la quantité de rayonnement reçu a été plus élevée;

3. Il n'existe aucun argument permettant de penser que cette déviation (si elle est réelle) soit dûe à une sensibilité particulière des spermatozoïdes, l'immense majorité des enfants recensés étant nés de gamètes irradiés au stade spermatogonial.

III. Sex ratio de la descendance des femmes irradiées

La descendance des 45 mères ayant eu des enfants avant et après traitement se répartit ainsi:

	♂	♀		Sex ratio
avant	37	24	= 61	0,606±0,07
Après	26	25	= 51	0,510±0,07

et la descendance de *toutes* les femmes:

	♂	♀		Sex ratio
Avant (154 mères)	130	106	= 236	0,551±0,034
Après (97 mères) dose moyenne de r = 1.360 .	63	73	= 136	0,463±0,044

Le déficit observé de la sex ratio après traitement n'est pas statistiquement significatif, mais il se produit dans le sens attendu le χ^2 est de 2,74 soit P = 0.10. Ici également quoique les chiffres soient bien faibles il est possible d'étudier la répartition des sexes en fonction de la taille de la fratrie après traitement.

Taille de la fratrie	1	2	3	4	5
♂	24	17	11	12	0
♀	24	30	12	7	0

Le déficit porte essentiellement sur les femmes qui n'ont eu que 2 enfants.

La répartition en fonction du temps est comparable à celle observée précédemment dans la descendance des hommes traités.

Armées	0–1	2–3	4–5	6–7	8 et plus
♂	8	16	15	10	14
♀	12	15	14	14	18

Si l'on admet l'éventualité de mutations liées au chromosome X il n'existe aucune raison de penser que l'effet génétique est plus marqué juste après le traitement que peu après. Cependant, ce découpage des données amenant à étudier des effectifs très restreints, aucune valeur statistique ne peut être accordée à ces résultats.

Discussion

Il faut tout d'abord reconnaître que l'hétérogénéité des échantillons tant des femmes que des hommes d'une part, que le petit nombre d'observations qui ont pu être recueillies d'autre part, diminuent considérablement la signification générale de la présente enquête.

Cependant comme une recherche de ce genre représente la seule possibilité actuelle d'évaluer directement les risques génétiques pour l'homme d'une irradiation des gonades, il est au moins permis d'essayer d'en tirer le maximum d'information.

Si nous considérons globalement les sex ratios de la descendance avant tout traitement des hommes traités par radiothérapie pelvienne, sans protection des gonades (pour névralgie sciatique et pour des motifs divers), et celle des femmes traitées par radiothérapie pelvienne, nous obtenons le tableau suivant:

Avant traitement

	♂	♀		Sex ratio
Hommes	358	338	= 696	0,514±0,019
Femmes	130	106	= 236	0,551±0,034

$\chi^2 = 0{,}962$ $\quad 0{,}50 > P > 0{,}30$

Ces deux descendances d'hommes et de femmes avant traitement pouvant donc être considérées comme homogènes il est alors possible de comparer leurs descendances après le traitement d'où le tableau

	♂	♀	Sex ratio
Hommes sciatiques	157	118 = 275	0,571±0,030
Total hommes (sciatiques + variés)	225	180 = 405	0,555±0,026
Femmes	63	73 = 136	0,463±0,044

La différence entre les sex ratios des enfants des femmes traitées et celle des hommes traités pour sciatique est significative: $\chi^2 = 4{,}24$ c'est-à-dire $0{,}05 > P > 0{,}02$.

Cependant la comparaison, femmes traitées contre total hommes traités n'atteint pas le seuil de 0,05: $\chi^2 = 3{,}49$. $P \# 0{,}06$.

Par contre, si nous sélectionnons la descendance des hommes ayant reçu plus de 1.000 r sur la région lombo-sacrée et la comparons à la descendance des femmes, la différence de la sex ratio après irradiation redevient significative, on a alors:

pour les hommes . . . . . 140 ♂ contre 104 ♀
et chez les femmes . . . . . 63 ♂ contre 73 ♀
$\chi^2 = 4{,}15$ $0{,}05 > P > 0{,}02$.

Il ressort de ces comparaisons que d'après nos données il est extrêmement vraisemblable que la sex ratio soit augmentée dans la descendance des hommes et abaissée dans la descendance des femmes du fait de l'irradiation.

Cependant, cette conclusion ne peut être formelle car ces comparaisons ne nous mettent pas à l'abri d'éventuelles distorsions dues principalement à l'existence de fratries monosexuées, un peu plus fréquentes que ne le voudrait le hasard seul, ainsi qu'on le sait.

En limitant les chiffres au premier enfant né après traitement on obtient en effet:

		1er enfant né après traitement ♂	♀	sex ratio
Pères	Sciatiques	102	76	0,573±0,037
	Motifs variés	39	42	0,482±0,055
Mères		37	44	0,457±0,055

La comparaison entre la descendance des femmes et celle des hommes traités pour sciatique donne $\chi^2 = 3{,}04$ c'est-à-dire $0{,}10 > P > 0{,}05$. Par contre si l'on utilise le total des hommes, le χ^2 s'abaisse à 1,49.

Cette diminution de la disparité des échantillons lorsque la comparaison est limitée au premier enfant, tient surtout au fait de fratries à masculinité élevée au 2°, 3° et 4° rang de naissance dans l'échantillon de motifs variés.

En conclusion les troubles de la sex ratio observés dans la descendance de femmes et d'hommes traités par radiothérapie pelvienne sont en bon accord avec l'hypothèse de l'induction de mutations dominantes léthales liées au chromosome X dans la descendance des hommes et de l'induction des mutations récessives léthales liées au sexe dans la descendance des femmes soumises au même traitement. Etant données les incertitudes relatives de notre échantillonnage et la petitesse des chiffres absolus, les faits rapportés ne peuvent constituer une preuve formelle de la production de mutations chez l'homme par l'énergie ionisante mais cette dernière hypothèse peut être considérée comme la seule explication «probable» des particularités observées.

La généralisation d'une telle enquête aux centres mondiaux susceptibles de fournir une information équivalente, selon un protocole commun, permettrait presque à coup sûr de réunir les données nécessaires à une conclusion définitive et à une première mesure de l'intensité du phénomène.

Résumé

Sur 238.800 dossiers dépouillés 4.428 cas d'irradiation pelvienne sur des adultes jeunes ont été relevés comprenant 3.579 hommes et 849 femmes.

Une enquête par questionnaire a permis d'établir la descendance de 517 hommes traités pour névralgie sciatique (irradiation lombo-sacrée dose moyenne 1.295 r) et de 269 cas d'irradiation pour des motifs variés, sans protection des gonades (dose moyenne 1.461 r).

Par ailleurs la descendance de 284 femmes, ayant reçu en moyenne 1.360 r a pu être enregistrée.

L'augmentation de fréquence des mort-nés, des fausses-couches et des malformés après traitement n'est pas envisagée en raison du petit nombre des observations et des imprécisions des réponses.

L'étude de la sex ratio révèle chez les hommes:

1. Un accroissement après traitement: 0,514 $\pm$0,019 avant et 0,555 $\pm$0,026 après R.X.
2. Une augmentation d'autant plus forte que la quantité de roentgens a été plus élevée: avant traitement 0,514 $\pm$0,019, après moins de 1000 r ($\bar{r}$ = 721) 0,528 $\pm$0,039: avant plus de 1000 r ($\bar{r}$ = 1.730) 0,574 $\pm$0,032.
3. Une variation indépendante du délai de conception après le traitement, ce qui permet d'inférer que si des mutations se sont produites, elles ont été induites dans les spermatogonies.

Chez les femmes on observe un abaissement de la sex ratio après traitement : avant traitement 0,551 ±0,034, après traitement 0,463 ±0,044.

Toutes ces déviations prises séparément ne sont pas statistiquement significatives. Cependant la comparaison de la descendance des pères irradiés et de celles des mères irradiées révèle une hétérogénéité à la limite de signification statistique. ($\chi^2 = 4{,}24$ pour $\nu = 1$.) Mais on ne peut éliminer un effet possible des fratries monosexuées.

Toutes ces constatations sont en bon accord avec l'hypothèse de mutations léthales induites sur le chromosome X, les dominantes étant décelées dans la descendance d'hommes et les récessives dans celle des femmes.

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INTEGRATED EXPOSURE FROM RADIOACTIVE FALLOUT AS A BASIS FOR ESTIMATING GENETIC EFFECTS

By A. E. BRANDT

The purpose of this paper is to present estimates of the integrated or infinity gamma ray exposure or dose for a few communities in the United States. These gamma doses are estimated indirectly from samples of fallout which are collected at various locations. Only those sampling stations within Continental United States will be discussed.

The fallout samples are collected on adhesive coated acetate film of which one square foot is exposed horizontally about three feet above the ground on a metal stand. The films are replaced daily at a given time. The exposed films and field data card are mailed to the Health and Safety Laboratory where the radioactivity of the collected fallout

material is assayed. Since the number of samples received daily is large, assembly line methods have been developed by the Laboratory for processing them. One important feature of this development is an automatic Beta Counter.

When a sample is received in the Analytical Laboratory it is ashed under specified conditions. The ash is placed in a plastic planchette which is then sealed between two strips of plastic and wound on a reel for processing in the automatic counter. Each reel when filled carries seventy planchettes sealed between strips in this manner. The first and last planchette on each reel are empty and serve to establish the background correction for the counts obtained on the other planchettes. Three of these other planchettes contain a known weight of potassium carbonate and are used as standards. From the recorded counts of these standards and the known K^{40} activity, the counts of the unknown samples can be converted to disintegrations per minute per square foot on the day counted.

Given the age of a sample when collected, its age when counted, its decay rate, and the Beta activity; an indirect estimate of the cumulative gamma dose due to external radiation may be made. The procedure for estimating this infinite gamma dose was developed by Dr. *John H. Harley* of this laboratory and his assistant Miss *Naomi A. Hallden.*

In an unpublished report, "Radioactive Fallout Through September 1955", dated June 13, 1956, *Merril Eisenbud* and *John H. Harley*, in discussing this procedure for estimating the gamma dose, say: "In calculating the dose, it has been assumed that the daily fallout is deposited uniformly on a infinite smooth plane where it remains to infinite time. The integrated infinite dose for each daily fallout is calculated from the measured beta activity, using known isotopic composition of the sample and the known gamma characteristics. The sum of these integrated infinite doses from each of the daily samples collected during any given time represents the estimated gamma dose for infinite time delivered to populations who are exposed from the start of the sampling period."

Continental United States has been divided into four regions for sampling fallout. Samples have been collected at seventy-one locations in Region 1, which includes most of the United States east of the Mississippi River; at forty-nine locations in Region 2, which includes the area between Region 1 and the Rocky Mountain States; at thirty-four points in Region 3, which consists of the Rocky Mountain States; and at twenty-four locations in the Pacific Coast States.

The gamma dose estimates in this paper are based on data for thirty-six months from October, 1952 to September, 1955, inclusive. The numbers of stations for which data were available for estimates covering the entire thirty-six months, the accumulated highest and lowest gamma exposures for the period, the modal class, and the number of stations in that class are given in Table 1.

At most of these stations provision was made for duplicate samples to be collected daily during parts of the period covered by this report. In no case, however, does the record show duplicate samples for every day of the sampling period. One sampling station has been selected from each region as a basis for a look at the sampling error. In each region the station with the highest exposure was selected if duplicates were available for a third or more of the period. If the station with the highest exposure did not have enough days with duplicates, the next station down the

Table 1. Integrated Gamma Doses in U.S. by Regions.

Region	Number of Stations	Accumulated Gamma Dose in Milliards			
		Max.	Min.	Modal Class	Number in Modal Class
I	36	30.9	4.9	7.0 to 11.0	22
II	30	28.7	7.2	16.0 to 20.0	11
III	28	76.7	8.7	7.0 to 17.0	9
IV	13	6.9	3.6	No distinct modal dose	
All	107	76.7	3.6	8.0 to 13.0	37

array that would satisfy this criterion was selected. In Regions I and III the station with the highest exposure qualified, the station with the second highest exposure qualified in Region II, and in Region IV the station with the next to the lowest exposure qualified but the exposures of all thirteen stations in this group were nearly equal.

Since the estimate of the integrated gamma dose is based on counts it is to be expected that the standard errors of such estimates will be correlated with the mean, for the standard deviation of a count is the square root of this count. The point to be made here is that the high exposure values are more precisely determined than the low ones.

A basis for estimating the potential genetic effect in the United States from radioactive products of weapons testing if the present rate of production (of fission products) is maintained over a period of thirty years is presented in Table 2.

Table 2. Possible Thirty Year Dose Based on Three Years of Data.

Region	Gamma Dose in Rads	
	From	To
I	.05	.31
II	.07	.29
III	.09	.77
IV	.04	.07

It is rather unlikely that any individual of reproductive age residing in the vicinity of any of the sampling stations covered by this study will absorb the entire gamma dose indicated by these fallout figures. However, they should be looked upon as representing the potential mutagenic force.

The real problem of evaluating the genetic effects of this radioactive fallout remains to be solved. Figures such as presented here must be transformed into mutation rates and further evaluated in terms of numbers of persons of reproductive age exposed to these forces before genetic effects on individuals and on a population as a whole can be assessed.

Discussion

A.E. Brandt (New York): Information on internal radiation resulting from the fallout of mixed fission products resulting from bomb tests has not been presented nor can an estimate be made from the external doses which have been given. However, an estimate of the accumulation of strontium 90 from October, 1952, through September, 1956 will be found in the final report on this period. In any case, the estimate of potential dose to the gonads due to the internal accumulation of strontium 90 from radioactive fallout is minor as compared to the external doses presented in this paper.

Eve, I. S.: Acta genet. *6*, 220–221, 1956

STATEMENT MADE BY WHO BEFORE THE INTERNATIONAL CONGRESS OF HUMAN GENETICS

By I. S. EVE

WHO, Geneva, Switzerland

On behalf of the World Health Organization I should like to ask the opinion of the Congress on a matter referred to us by the United Nations Scientific Committee on the Effects of Atomic Radiation. This Scientific Committee was convened by the General Assembly of the United Nations with terms of reference such as "to receive and assemble in an appropriate and useful form ..... reports on scientific observations and experiments relevant to the effects of ionizing radiation upon man and his environment".

In the unpublished conclusions of the first session of the United Nations Scientific Committee there is a section on the scope of the work of the Committee on genetics. This recommended that material should be collected concerning work on such questions as natural mutation rates and changes in mutation rates after different radiation dosages. Information was to be obtained regarding local conditions giving opportunities for studies of populations in areas with different levels of natural background of ionizing radiation. Information and proposals were also to be collected about phenomena which might be used as genetic indicators in human genetic work over large areas.

A further suggestion was as follows: "This year the human geneticists will meet at a congress of human genetics. This opportunity should be used, with the assistance of the World Health Organization, to seek advice about the possibility of setting up a standard of recognition for one or more clearly recognizable medical conditions thought to be largely or solely genetic in origin."

I should be very glad, if members of the Congress have views on this subject, if they could make suggestions on the most appropriate medical conditions for such studies. As WHO is holding a Study Group after the Congress, which will include consideration of this topic on its agenda, it will be able to word a reply to the U N Scientific Committee, on the basis of the views which the Congress expresses on the subject.

We should very much appreciate your co-operation.

RESOLUTION OF THE FIRST INTERNATIONAL CONGRESS OF HUMAN GENETICS ON THE SUBJECT OF RADIATION DAMAGE

in response to a request by Dr. Eve of WHO for an expression of the viewpoint of congress members on this subject

The damage produced by ionizing radiation on the hereditary material is real and should be taken seriously into consideration in both the peaceful and military uses of nuclear energy as well as in all medical, commercial and industrial practices in which X-rays or other ionizing radiation is emitted. It is recommended that the investigation of the amount and type of damage and of related genetic questions, be greatly extended and intensified with a view to safeguarding the wellbeing of future generations.

EXPERIMENTAL PATHOLOGY AND CYTOLOGY IN RELATION TO HUMAN GENETICS

Nachtsheim, H.: Acta genet. *6*, 223–239, 1956

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VERGLEICHENDE UND EXPERIMENTELLE ERBPATHOLOGIE IN IHREN BEZIEHUNGEN ZUR HUMANGENETIK

Von H. NACHTSHEIM

Das Bild der Humangenetik unterliegt in jüngster Zeit einem starken Wandel. In den ersten Jahrzehnten nach der Wiederentdeckung der *Mendel*schen Gesetze suchte man ihre Gültigkeit auch für den Menschen zu erweisen, indem man eine Fülle von Material über den Erbgang möglichst vieler normaler und vor allem krankhafter menschlicher Merkmale sammelte. Im großen und ganzen bewegte man sich indessen im Schlepptau der experimentellen Genetik, deren Vertreter die Bemühungen der Humangenetiker vielfach als Versuche am untauglichen Objekt betrachteten. Aber ist es verwunderlich, wenn z. B. ein Meister des Experiments wie *Thomas Hunt Morgan*, ohne dessen Entdeckung der Drosophila als Versuchstier der Genetik die Entwicklung unseres Forschungsgebietes in so kurzer Zeit bis zu seiner heutigen Höhe einfach undenkbar wäre, immer etwas mitleidig auf die Humangenetiker sah? Zwar hat er das Thema «Menschliche Vererbung und moderne Genetik» mehrfach in Vorträgen selbst behandelt, doch merkt man bisweilen die leichte Selbstironie, mit der er es tut. Nicht nur den Menschen, auch die Säugetiere hielt *Morgan* für Objekte, mit denen sich grundlegende Beiträge zur Genetik nicht liefern lassen. Ich erinnere mich lebhaft eines Gespräches mit ihm vor genau 30 Jahren. Es kam die Rede auf einen bekannten amerikanischen Genetiker. "He is a first-class man", sagte *Morgan*, "but he should not work on mammals".

Nun, in den seither vergangenen drei Jahrzehnten haben sich unsere Anschauungen über die Brauchbarkeit biologischer Objekte zu erb-

analytischen Untersuchungen wesentlich geändert. Nicht nur die höchsten, auch die niedersten Organismen sah man zunächst als ungeeignet für den Erbforscher an. Heute jedoch ist die Mikrobengenetik dank neu entwickelter Methoden zu einem der fruchtbarsten und aussichtsreichsten Zweige der Erbforschung geworden. Ebenso steht die Humangenetik im Begriffe, ein, wie ich es nennen möchte, eigenes Gesicht zu gewinnen. Sie begnügt sich nicht mehr mit einer bloßen Materialsammlung und einer einfachen Bestätigung der Befunde der Experimentalforscher am Menschen, sie entwickelt ihre besondere Problematik, schafft sich eigene Methoden für die Inangriffnahme ihrer Probleme und bemüht sich mit Erfolg, auch zur Grundlagenforschung wertvolle Beiträge zu liefern. Wie sehr die Wertschätzung der Humangenetik als Forschungsgebiet gestiegen ist, mag daraus ersehen werden, daß sich einige unserer bedeutendsten Experimentalforscher und Drosophilagenetiker – und gerade solche aus dem ehemaligen Arbeitskreis von *Morgan*, ich nenne nur *Muller*, *Dobzhansky* und *Stern* – in jüngster Zeit auch der Humangenetik zugewandt haben.

So begrüßenswert die selbständige Entwicklung der Humangenetik aber auch ist, so darf sie doch keinesfalls zu einer Isolierung dieses Forschungsgebietes führen. Im Gegenteil ist eine um so engere Zusammenarbeit mit der vergleichenden und experimentellen Forschung zu erstreben. Die Ergebnisse der Humangenetik müssen an denen der allgemeinen Genetik überprüft und durch sie ergänzt werden. Die wichtigste Methode der allgemeinen Genetik ist und bleibt die Analyse des Genotypus vermittels des Kreuzungsexperimentes. Beim Menschen steht uns diese Methode nicht zu Gebote. Im Modellversuch am Tier haben wir aber die Möglichkeit, viele Fragen, die die Erforschung am Menschen offen läßt, zu beantworten.

Die dem Menschen am nächsten stehenden Lebewesen sind die Säugetiere, und unter ihnen eignen sich für den Modellversuch wiederum aus rein technischen Gründen ganz besonders die als Versuchstiere sprichwörtlich gewordenen Nagetiere, Kaninchen und Meerschweinchen, Ratte und Maus. Man hört bisweilen, besonders aus humanmedizinischen Kreisen, die zweifelnde Frage, ob denn nicht der Abstand der Ordnung Primaten im System von der Ordnung Nager, wenn auch beide nach dem gleichen Bauplan konstruierte Säuger sind, doch zu groß sei, um am Nager gewonnene Befunde auf den Menschen zu übertragen und Parallelen zu ziehen. Gewiß haben die Nager viele art-, gattungs-, familien*spezifische* Erbmerkmale, und der Genotypus Kaninchen z. B. ist aus den Genmolekülen anders aufgebaut als der Genotypus Mensch, überdies in einem

anderen Substrat wirksam. Das unterschiedliche *Gengefüge* schließt indessen nicht aus, daß Kaninchen und Mensch zahlreiche *Einzelgene* gemeinsam haben, daß diese homologen Gene in entsprechender Weise mutieren und Allelenserien bilden und daß die gleichen Genwirkketten zu entsprechenden Endprodukten führen können. Inwieweit die Endprodukte der beiden Vergleichsobjekte dann in ihrem Erscheinungsbild voneinander abweichen, hängt von den artspezifischen Besonderheiten der beeinflußten Organe ab, ganz allgemein gesagt, von den Differenzen der Gengesellschaft, in der sich die homologen Gene befinden, und von dem Substrat, in dem die Gene sich auswirken. Selbstverständlich muß eine kritiklose Gleichsetzung klinischer Symptome und Krankheitsbilder von Mensch und Säugetier vermieden werden. Der mit diesen Objekten vergleichend arbeitende Forscher muß mit den Besonderheiten *beider* vertraut sein, um keine falschen Parallelen zu ziehen und Fehlschlüssen zu entgehen.

Doch betrachten wir an einigen Beispielen, die teilweise den eigenen Arbeiten entnommen sind, die Mittel und Wege, auf denen die vergleichend-experimentelle Erbpathologie Beiträge zu Fragen der Humangenetik zu liefern vermag, die diese mit ihren Methoden nicht zu beantworten imstande ist. Anschließend soll dann, soweit es die Zeit gestattet, noch ein Hinweis auf Problemkreise gegeben werden, die, wie uns scheint, in nächster Zukunft in besonderem Maße der experimentellen Bearbeitung bedürfen.

Das erste Beispiel soll die Abhängigkeit des Schicksals einer Mutation von der Spezies demonstrieren, in der das mutierte Gen auftritt. Die gleiche Mutation kann in der einen Spezies durchaus vitale Individuen liefern, während sie sich in einer anderen Spezies infolge bestimmter Besonderheiten in der Organisation letal auswirkt. Dabei ist keineswegs das höchstorganisierte Lebewesen, der Mensch, stets am meisten gefährdet.

In seinem bekannten Buche «Die körperliche Grundlage der Persönlichkeit» gibt *Ch. R. Stockard* [1932] als Titelbild (Abb. 1) einige Parallelen zwischen Mensch und Hund wieder, ein paar innersekretorisch bedingte Erbtypen, die beim Menschen sippenmäßig gehäuft vorkommen, beim Hund sogar als Rassetypen konstant gezüchtet werden. Zu den wichtigsten Rassenmerkmalen der in der oberen Reihe dargestellten Bulldogge gehört die Brachygnathia superior, die Verkürzung des Oberkiefers. Der Unterkiefer muß den Oberkiefer überragen und deutlich nach oben aufgebogen sein. Der Japan-Chin in der unteren Reihe zeichnet sich ebenfalls durch eine stark zusammengestauchte Gesichtspartie aus, die, wie bei

Abb. 1. Vergleich zwischen Hunderassen und Menschentypen. Obere Reihe: Brachygnathia superior; mittlere Reihe: Akromegalie; untere Reihe: chondrodystropher Zwergwuchs. (Nach *Ch. R. Stockard* [1932].)

seinem nächsten Verwandten, dem Pekingesen, mit einem allgemeinchondrodystrophen Zwergwuchs verbunden ist. Abb. 2 zeigt die Schädel von 4 dieser brachygnathen Hunderassen. Die Mopsköpfigkeit wird als mutativ auftretendes Merkmal fast in allen Säugergruppen einschließlich des Menschen beobachtet. Handelt es sich bei der Brachygnathie um eine monosymptomatische Mutation, so beeinträchtigt sie die Lebensfähigkeit ihres Trägers nicht oder nicht wesentlich. Auch in einem Syndrom mit mehr oder weniger zahlreichen Symptomen ist die Brachygnathie des Oberkiefers in der Regel eine der harmlosesten Veränderungen, für die Letalität eines solchen Syndroms, wie z. B. des Bulldogkalbes, sind andere Anomalien verantwortlich. Diese Angaben über die Vitalität brachygnather Individuen gelten aber *nicht* für *eine* Gruppe von Säugern, für die *Nager*. Hier wirkt sich jede Brachygnathia superior, mag sie als mono- oder polysymptomatische Mutation auftreten, infolge der besonderen Beschaffenheit der Nagezähne letal aus.

Vor etwa 20 Jahren erhielt ich beim Kaninchen eine Mutation, bei der die Oberkieferverkürzung das einzige Symptom ist (Abb. 3). Streng genommen handelt es sich in diesem Falle nicht um eine Verkürzung,

Abb. 2 Abb. 3 Abb. 4

Abb. 2. Schädel brachygnather Hunderassen. Von oben nach unten Zwergbulldogge, Mops, Japan-Chin, Pekingese. (Nach *Nachtsheim* [1940].) – *Abb. 3.* Brachygnathia superior beim Kaninchen. Oben: normal; Mitte und unten: verschiedene Grade der Verkürzung des Oberkiefers und des Wucherns der Nagezähne. (Nach *Nachtsheim* [1940].) *Abb. 4.* Zwergwuchs beim Kaninchen. Schädel 20 Tage alter Tiere. In der Mitte Schädel eines gleichalten normalen Tieres zum Vergleich. (Nach *Schnecke* [1941].)

sondern um eine Aufwölbung des Schnauzenteiles des Schädels. In den ersten 4 Lebenswochen wächst der Schädel normal. Statt sich aber beim weiteren Wachstum ähnlich wie der Unterkiefer zu strecken, wölbt sich der Oberkiefer im Bereich des Diastemas nach oben. Auch auf die Nasalia überträgt sich diese Wölbung. Infolgedessen erscheint am Schädel dieser Tiere die Profillinie nicht gerade, sondern, wie auch die Abbildungen zeigen, etwas konvex. Diese erblich bedingte Kieferveränderung – es liegt ein einfach-rezessiv mendelndes Merkmal vor – wäre an sich harmlos, hat aber dadurch einen verheerenden Einfluß, daß sie die Stellung der für den Nager so wichtigen Nagezähne verändert. Während beim normalen

Kaninchen die keilförmig zugeschliffenen Nagezähne beim Kieferschluß so liegen, daß die oberen Inzisiven über die unteren greifen, stoßen beim Zurückbleiben der Längenentwicklung des Oberkiefers hinter der des Unterkiefers die Inzisiven aufeinander, wodurch ihre Funktion bereits stark beeinträchtigt wird; die keilförmige Abnutzung geht verloren, die Zähne werden an ihren oberen Enden flach wie Backenzähne. Geht die Hemmung des Längenwachstums des Oberkiefers noch weiter, so kommen die Nagezähne in umgekehrte Stellung wie normal, die unteren Inzisiven übergreifen jetzt die oberen. Das bedeutet völlige Außerfunktionssetzung. Da aber die Zähne der Nager im Gegensatz zu den meisten übrigen Säugerzähnen wurzellos sind und zeitlebens wachsen, müssen die funktionslosen Nagezähne in Kürze überlang werden. So wächst einem Tier mit einer solchen Kiefer- und Zahnanomalie das Maul innerhalb weniger Wochen regelrecht zu, es muß verhungern. Zwar kann man die Tiere vor dem Verhungern bewahren, indem man ihnen die wuchernden Nagezähne alle paar Wochen abkneift. Man kann dann die Tiere mühsam mit Weichfutter aufziehen und zur Fortpflanzung bringen und so theoretisch einen reinen Stamm mit Brachygnathia superior gewinnen, doch bleibt es dem Züchter infolge der Besonderheiten im Bau der Nagezähne versagt, eine Rasse Mops- oder Bulldogkaninchen zu züchten.

In einem anderen von mir gezüchteten Kaninchenstamm mit Brachygnathia superior ist die Oberkieferverkürzung eines von vielen Symptomen im Syndrom eines einfach-rezessiv erblichen Zwergwuchses (Abb. 4). Hier ist der verkürzte Oberkiefer schon von Geburt an vorhanden, und die falsche Stellung der Nagezähne macht bereits das Saugen unmöglich, mit spätestens drei Wochen gehen die Jungen zugrunde.

Auch bei anderen Nagern haben Mutationen dieser Art stets früher oder später eine letale Wirkung.

Diesem organisationsbedingten unterschiedlichen Verhalten von Nager und Mensch seien einige Beispiele gegenübergestellt, in denen die Übereinstimmung in die Augen fällt.

In der Nachkommenschaft röntgenbestrahlter Mäusemännchen erhielt *Paula Hertwig* [1939, 1940] eine einfach-rezessiv erbliche Mutation, die als Leitsymptom eine Oligodaktylie aufweist. *Hedwig Freye* [1954], der wir eingehende anatomische und entwicklungsgeschichtliche Untersuchungen an den oligodaktylen Mäusen verdanken, stellte die folgenden Symptome am Skelett fest (Abb. 5): Reduktion der Finger- und Zehenstrahlen, Verschmelzungen und Ausfall im Carpus bzw. Tarsus, mehr oder weniger weitgehende Aplasie von Ulna und Fibula bis zu deren vollständigem Fehlen, Hemimelie der hinteren Extremitäten, Fehlen des

Abb. 5. Oligodaktylie bei der Maus. Neugeborene, oben normales Tier; unten mit Oligodaktylie, Alizarinfärbung. (Nach *Freye* [1954].)

13. Rippenpaares. Fusion der Sternalsegmente. Knickschwanz, Kurzschwanz. Die vorderen Gliedmaßen sind häufiger und stärker fehlgebildet als die hinteren. Die linke Seite ist stärker betroffen als die rechte. Außer den Skelettanomalien haben die oligodaktylen Mäuse eine verkleinerte Milz. Nierenaplasie. Hufeisen- oder Zystenniere, wobei wiederum die linke Seite häufiger befallen ist als die rechte. Die Sterblichkeit ist unter den befallenen Tieren groß, alle am Leben erhaltenen Oligodaktylen, ♂♂ und ♀♀, sind sehr schwächlich und bleiben steril, die Geschlechtsorgane sind unterentwickelt.

Zu diesem Oligodaktylie-Syndrom der Maus gibt es beim Menschen ein Gegenstück, das in jüngster Zeit von *H. Weyers* an mehreren Fällen näher untersucht wurde. Da Herr Dr. *Weyers* sein Bildmaterial in der Ausstellung dieses Kongresses demonstriert, möchte ich ihm nicht vorgreifen und begnüge mich mit diesem Hinweis. In Tabelle 1 habe ich die

Tabelle 1. Gleiche Symptome im Oligodaktylie-Syndrom bei Maus und Mensch.

± Aplasie von Ulna und Fibula bis zu gänzlichem Fehlen.
Verschmelzung und Ausfall im Carpus (und Tarsus).
Vordere Extremitäten häufiger und stärker befallen als hintere.
Reduktionsvorgänge links häufiger und stärker als rechts.
Fixierung der Armknochen in Ellenbeuge.
Sternalsegmente ± verwachsen.
Dystopische Nieren, Hufeisen-, Zystenniere.
Anomalien der Nieren links häufiger als rechts.

Abb. 6. Dystrophia muscularis bei der Maus. Links krankes, rechts normales Tier, $4^1/_2$ Monate alt. (Nach *Michelson, Russell* und *Harman* [1955].)

wichtigsten Symptome des Syndroms zusammengestellt, die bei Maus und Mensch übereinstimmen.

Es ist *Weyers* gelungen, einschließlich seiner eigenen Beobachtungen in der Literatur 12 Vollsyndrome aufzufinden, die bis auf das Jahr 1683 zurückgehen. Die meisten sind sporadische Fälle, doch liegen auch Familienbeobachtungen an mehreren Generationen vor, ohne daß bis heute eine klare Beurteilung des Erbganges möglich ist. Mit dem Vorkommen von Neumutationen oder nicht-erblichen Phänokopien muß ebenfalls gerechnet werden. Beim Menschen scheint das Oligodaktylie-Syndrom weniger letale Wirkung zu haben als bei der Maus.

Eine weitere Mutation bei der Maus, der, wie mir scheint, für die vergleichende und experimentelle Bearbeitung besonders große Bedeutung zukommt, ist kürzlich von *A. M. Michelson, E. S. Russell* und *P. J. Harman* [1955] beschrieben worden. Es handelt sich um den ersten Fall einer erblichen primären Myopathie bei der Maus und bei Nagern überhaupt. Die Dystrophia muscularis der Maus entspricht in ihrem Krank-

Abb. 7. 2 Monate alte Muskeldystrophie-Maus, ♂, mit starker Kyphose. (Nach *Michelson, Russell* und *Harman* [1955].)

heitsbild und ihrer Histologie weitgehend der Dystrophia musculorum progressiva des Menschen, und zwar der sog. aufsteigenden oder Beckengürtelform, die beim Menschen in zwei Erbtypen, einem mit geschlechtsgebunden-rezessivem und einem mit autosomal-rezessivem Erbgang, vorkommt. Bei der Maus ist der Erbgang einfach-rezessiv.

Die Krankheit wird bei der Maus im Alter von $3\frac{1}{2}$ Wochen erkennbar, Zeichen von Ataxie und gelegentlich einseitige Parese der Hinterbeine treten auf, später ist die Parese bilateral. Abb. 6 zeigt die verkrampfte Haltung der Hinterbeine, wenn das kranke Tier (links) am Schwanz hängend gehalten wird, während das normale Tier (rechts) mit den ausgestreckten Hinterbeinen strampelnde Bewegungen macht. Mit der Parese setzt die Muskelatrophie ein, die, beginnend an den Hinterbeinen, über die axiale Muskulatur zu der der Vorderbeine fortschreitet. Das 2 Monate alte Tier der Abb. 7 mit starker Kyphose hat die Funktionskontrolle über die Hinterbeine verloren, diese werden nachgeschleppt, die Fortbewegung ist nur noch vermittels der atrophischen Vorderbeine möglich. Der Tod tritt auf verschiedenen Stadien der Krankheit ein, meist zwischen 1–6 Monaten, spätestens mit 8–9 Monaten. Auch die das geschlechtsreife Alter erreichenden Individuen sind physisch zur Paarung unfähig, wenn auch die Gonaden normal entwickelt sind. Die Funktionsfähigkeit der Ovarien konnte durch Transplantation in gesunde Mäuse nach Ovariektomie und Paarung dieser Wirtsweibchen mit gesunden Männchen bewiesen werden. Alle so erhaltenen Nachkommen waren

heterozygot im Muskeldystrophie-Gen und lieferten bei Paarung mit den Heterozygoten des Myopathie-Stammes die erwartete Spaltung nach dem 3 : 1-Verhältnis.

Aus den histologischen Untersuchungen sei erwähnt, daß im neuralen Gewebe keine pathologischen Veränderungen gefunden wurden. Das Muskelgewebe zeigt eine Proliferation der Sarkolemmkerne, eine Vermehrung des interstitiellen Gewebes, Größenvariationen der einzelnen Muskelfasern, die ein Gemisch aus pathologischen und normalen Fasern darstellen, Beobachtungen, die mit den pathologischen Kriterien bei den menschlichen Muskeldystrophien übereinstimmen.

Wir dürfen wohl mit Sicherheit damit rechnen, daß die Fortsetzung dieser erst am Anfang stehenden Untersuchungen an der Muskeldystrophie der Maus bei den vielseitigen experimentellen Möglichkeiten noch für das Verständnis der Ätiologie der menschlichen Muskeldystrophien und ihre Therapie wichtige Ergebnisse zeitigen wird.

In den bisher gewählten Beispielen handelte es sich beim Tier stets um rezessiven Erbgang. Dieser ist beim Tier weit häufiger als der dominante Erbgang, während es beim Menschen gerade umgekehrt ist, wozu freilich gleich gesagt werden muß, daß hier nur ein scheinbarer Unterschied besteht. Beim Tier erfassen wir durch die weitgehend betriebene Inzucht die rezessiven Leiden in gleichem Maße wie die dominanten, beim Menschen entgehen uns, da Inzucht die Ausnahme ist, die rezessiven Leiden größtenteils, oder sie treten als sporadische Fälle auf, die oftmals trotz Familienuntersuchungen keine sichere Entscheidung über die Frage erblich oder nicht-erblich, geschweige denn den Nachweis des Erbganges gestatten. In meinem Referat bei dem letzten internationalen Genetiker-Kongreß (1953) habe ich nach dem damaligen Stand eine Gegenüberstellung der Häufigkeit der verschiedenen Erbgänge bei den wichtigsten Versuchstieren einerseits, dem Menschen andererseits gegeben. Während bei allen Säugetieren die rezessiven Leiden stark überwiegen, werden die autosomal bedingten Erbleiden beim Menschen zu etwa 75% dominant vererbt. Lediglich hinsichtlich der in den Geschlechtschromosomen lokalisierten krankhaften Gene stimmen Tier und Mensch in der Häufigkeit von Dominanz und Rezessivität überein, was wiederum durchaus verständlich ist, da ja die Herausspaltung der geschlechtsgebunden-rezessiv erblichen Leiden auch ohne Inzucht erfolgt.

Je seltener ein rezessives Leiden beim Menschen ist, um so schwieriger ist seine Erbanalyse. Beim Versuchstier gibt es keine seltenen Erbleiden. Sobald wir die Erbkrankheit einmal erfaßt haben, können wir die Merkmalsträger in beliebiger Individuenzahl züchten und in allen Ent-

wicklungsstadien lebend und tot untersuchen. Selbst wenn die Merkmalsträger sich auf früherem oder späterem Entwicklungsstadium als letal oder doch als fortpflanzungsunfähig erweisen, können wir das krankhafte Gen über die Heterozygoten weiterzüchten und immer wieder neues Untersuchungsmaterial gewinnen, wenn dieser Weg auch mühsam ist, da er immer aufs neue die Prüfung der Zuchttiere auf Heterozygotie erfordert. Ich habe Kaninchenstämme mit bestimmten rezessiven Erbleiden, deren krankhafte Gene auf diese Weise über mehrere Jahrzehnte erhalten werden konnten.

Auch die Analyse dominanter Erbleiden bietet beim Menschen Schwierigkeiten, die wir beim Tier nicht kennen. Die meisten der 75% autosomal-dominanter Erbleiden des Menschen sind uns nur in heterozygotem Zustand bekannt, über das Aussehen der Homozygoten, ob sie lebensfähig oder letal sind, wissen wir nichts oder nichts Sicheres. Als ein Beispiel für viele seien Spalthand und Spaltfuß genannt. Daß diese Mißbildung einfach-dominant vererbt wird, steht fest, aber noch nie ist m. W. eine Nachkommenschaft aus der Verbindung Spalthand × Spalthand beobachtet worden.

Seit kurzem besitzen wir einen Stamm Spalthandkatzen (Abb. 8). Die Ausgangstiere unserer Versuche verdanke ich Professor *Haldane*, in dessen Institut *A. G. Searle* [1953] bereits eine Untersuchung über die Erblichkeit des Merkmals durchgeführt hat. Wie beim Menschen erfolgt die Vererbung einfach-dominant mit etwas unregelmäßiger Manifestation. Auch im morphologischen Bild stellt die Spalthand der Katze eine vollkommene Parallele zu der Spalthand des Menschen dar. Ich kann diese Befunde durchaus bestätigen. *Searle* hat aber nur Heterozygoten untersucht. Da die Spalthandkatzen voll vital und zuchtfähig sind, bietet die

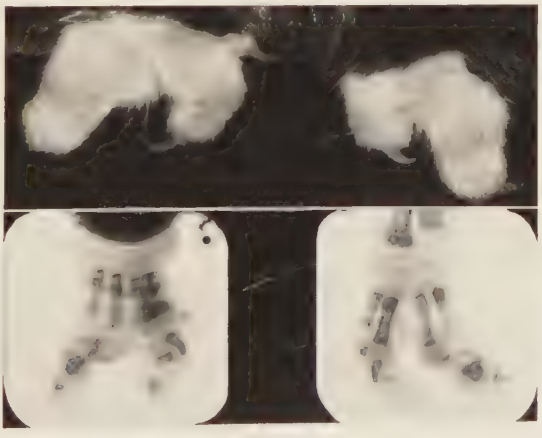

Abb. 8. Vorderpfoten einer Spalthandkatze, ♀, 3 Wochen alt. Unten Röntgenbilder der Vorderpfoten dieses Tieres. (Nach *Nachtsheim* [1957].)

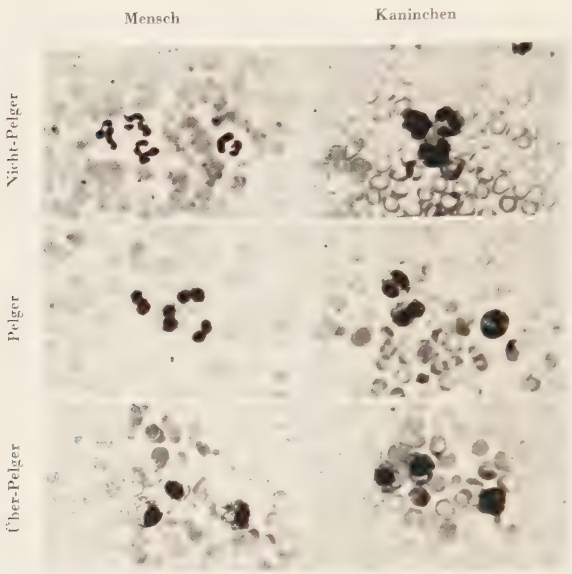

Abb. 9. Pelger-Anomalie bei Mensch und Kaninchen, neutrophiles Blutbild. In der Mitte heterozygote Individuen, unten homozygote Individuen, oben normale zum Vergleich. (Nach *Nachtsheim* [1954].)

Gewinnung der F_2-Generationen keine Schwierigkeiten. Wir haben in diesem Jahre auch bereits einige F_2-Würfe erhalten, doch möchte ich Aussagen über die Homozygoten zurückstellen, bis die F_2-Tiere auch genetisch geprüft sind.

In diesem Zusammenhang noch einiges zu dem – ich möchte sagen Paradebeispiel aus meinen Versuchen zur Pelger-Anomalie (Abb. 9). Die von dem holländischen Tuberkulosearzt *Pelger* [1928] beim Menschen entdeckte und 1939 von *E. Undritz* auch für das Kaninchen nachgewiesene einfach-dominant erbliche Anomalie besteht im wesentlichen in einer Hemmung des Segmentierungsprozesses der Neutrophilenkerne. Das Leitsymptom der heterozygoten Pelger sind die sogenannten Zwicker, die im reifen Zustand aus zwei Segmenten bestehenden Kerne. Die Anomalie stimmt bei Mensch und Kaninchen bis in alle Einzelheiten so weitgehend überein, daß ein begründeter Zweifel an ihrer Homologie kaum geäußert werden kann.

Die homozygoten Pelger waren für den Menschen bis vor kurzem unbekannt, beim Kaninchen konnte ich sie seit 1942 systematisch züchten. Die Hemmung der Kernsegmentierung ist bei den Homozygoten, den «Über-Pelgern», wie ich sie nannte, total: die Neutrophilenkerne bleiben einfach-rund. Ich sagte voraus, daß das Blutbild bei den homozygoten Pelger-Menschen ebenso beschaffen sein werde. Im übrigen aber erwiesen sich die homozygoten Pelger-Kaninchen im Gegensatz zu den sehr vitalen

heterozygoten Individuen als weitgehend letal. Die meisten Homozygoten zirka 85% gehen bereits frühembryonal zugrunde. Soweit sie diese erste Klippe überwinden, erreichen sie zwar die Geburt, sterben aber bald nach dieser, nur einige wenige Tiere konnten aufgezogen, ins geschlechtsreife Alter gebracht und als fertil nachgewiesen werden. Die homozygoten Pelger-Kaninchen zeigen, soweit sie erst bei der Geburt zugrunde gehen, das Bild einer hochgradigen Chondrodystrophie.

Um beurteilen zu können, wie häufig beim Menschen homozygote Pelger zu erwarten sind, müßte die Häufigkeit heterozygoter Pelger in der Population festgestellt werden. In Berlin fand ich bei Untersuchung von über 20 000 Individuen beider Geschlechter aller Altersstufen knapp 1 Pelger auf 1000 Individuen. So ist unter $1000 \times 1000 = 1$ Million Verbindungen einmal die Kombination Pelger × Pelger zu erwarten, und da bei dieser Kombination jedes 4. Kind ein homozygoter Pelger sein sollte, käme auf 4 Millionen Geburten theoretisch 1 Über-Pelger, die Lebensfähigkeit der Homozygoten wenigstens bis zur Geburt vorausgesetzt. Unter diesen Umständen war es schon ein besonderer Glückszufall, daß Frau *Haverkamp-Begemann*, eine holländische Kinderärztin, unter den Kindern zweier blutsverwandter Eltern (Vetter und Cousine) 1952 einen homozygoten Pelger, ein $2\frac{1}{2}$jähriges Mädchen, entdeckte. Erkannt wurde die Homozygotie an dem weißen Blutbild, das vollauf dem des homozygoten Pelger-Kaninchens entspricht, und anschließend wurden dann beide Eltern des Kindes als heterozygote *Pelger* nachgewiesen. Das körperlich und geistig stark zurückgebliebene und an epileptischen Anfällen leidende homozygote Pelger-Kind soll im Knochensystem normal sein, also frei von Chondrodystrophie. Leider fehlt ein eingehender klinischer Bericht. Ob die menschlichen homozygoten Pelger tatsächlich vitaler sind als die entsprechenden Kaninchen und frei von Veränderungen im Knochensystem, läßt sich nach dem einen Fall noch nicht endgültig beurteilen. Beachtlich ist indessen, daß von 9 Schwangerschaften der Mutter des homozygoten Pelger-Kindes 3 mit Aborten endeten, 3 Kinder starben früh. Blutuntersuchungen fehlen. 2 lebende Geschwister der Probandin sind Nicht-Pelger. Eine erneute und eingehende Untersuchung der Familie wäre dringend erwünscht.

Noch in anderer Hinsicht kann die Pelger-Anomalie für die vergleichende erbpathologische Forschung als beispielhaft gelten. Ich denke an das Thema Mutation und Phänokopie bei Mensch und Tier.

Es ist bekannt, daß das weiße Blutbild ein äußerst feiner Indikator für viele krankhafte Prozesse im Körper ist. Infektionen und Entzündungen führen zu sogenannten Linksverschiebungen, bestimmte Krank-

heiten rufen Rechtsverschiebungen hervor. Gerade diese starke Modifikabilität des weißen Blutbildes war der Grund, daß man die *Pelger*-Anomalie relativ spät entdeckte. Man verwechselte diese «erbliche Linksverschiebung», wie wir sie nennen können, mit exogen bedingten Veränderungen des Blutbildes, und diese Verwechslungen kommen sicher auch heute noch oftmals vor.

Das Pelger-Blutbild ist nun in ganz ähnlicher Weise modifizierbar wie das normale Blutbild, d.h. auch hier sind Links- und Rechtsverschiebungen möglich. Meine Mitarbeiterin *Helga Harm* untersuchte die Wirkung von Intoxikationen auf das Blutbild des Pelger-Kaninchens. Durch intravenöse Injektion von Colchicin konnte sie bei heterozygoten Pelger-Kaninchen eine Linksverschiebung erzielen und dadurch das Bild des heterozygoten in das des homozygoten Pelgers überführen (Abb. 10). Durch einen toxischen Reiz läßt sich also das Phän des Homozygoten am Phän des Heterozygoten phänokopieren, die Reizwirkung entspricht der Wirkung des fehlenden Pelger-Allels. Freilich ist diese induzierte Phänokopie nur transitorisch, sie schwindet mit dem Wegfall des Reizes.

Eine ganz ähnliche Phänokopie haben Frau *Lüers* und ich vor kurzem beim heterozygoten Pelger-Menschen beobachten können. Unter Blutausstrichen, die uns aus einem Ostberliner Krankenhaus übersandt worden waren, fanden wir zu unserer großen Überraschung ein Blutbild von einem 10jährigen Mädchen, das vollkommen dem des homozygoten holländischen Pelger-Kindes entsprach (Abb. 11). Sollte der zweite homo-

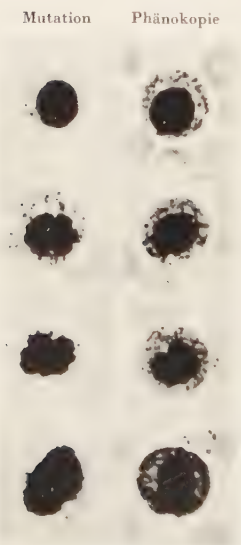

Abb. 10

Abb. 10. Links rundkernige neutrophile Leukozyten beim homozygoten Pelger-Kaninchen; rechts entsprechende Form beim heterozygoten Pelger-Kaninchen nach Colchicinbehandlung. (Nach *Harm* [1953].)

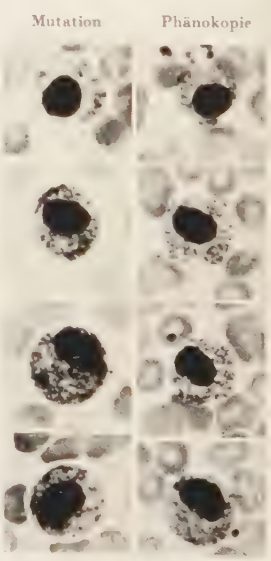

Abb. 11. Variationsbreite der reifen rundkernigen Neutrophilen beim Pelger-Menschen, links bei dem homozygoten Pelger-Kind Franziska N. (94 %); rechts bei dem heterozygoten Pelger-Kind Edeltraut H. während einer hochfieberhaften Erkrankung (72 %). (Nach *Lüers, Nachtsheim* und *Petzel* [1956].)

Abb. 11

zygote Pelger entdeckt sein? Die Familienuntersuchung lieferte den Beweis, daß das Mädchen aus einer Pelger-Sippe stammte, in der die Vererbung der Anomalie über drei Generationen verfolgt werden konnte. Die Mutter der Probandin erwies sich als heterozygoter Pelger, der Vater aber hatte ein normales Blutbild. Um einen homozygoten Pelger konnte es sich also bei der Probandin nicht handeln. Aus der Krankengeschichte ging jedoch hervor, daß das Kind zur Zeit der Herstellung des Blutausstriches an einer hochfieberhaften Angina litt. Wir untersuchten das Blutbild des Kindes anschließend an die Erkrankung wiederholt über einen längeren Zeitraum und konnten feststellen, daß mit der Genesung das Bild wieder zu dem des heterozygoten Pelgers zurückkehrte, es lag eine transitorische Phänokopie des homozygoten infolge reaktiver Linksverschiebung beim heterozygoten Pelger vor.

Unsere Beobachtungen sollten als eine Warnung dienen. Es besteht heute in Kreisen der Hämatologen und Kliniker vielfach die Neigung, beim Auffinden eines pelgerähnlichen Blutbildes ohne jede Familienuntersuchung kurzerhand von einem (heterozygoten) Pelger zu sprcehen. Während man *vor* Entdeckung der Pelger-Anomalie diese mit Linksverschiebungen verwechselte, kommt es heute vor, daß Linksverschobene fälschlich zu Pelgern deklariert werden.

Auch die Diagnose homozygoter Pelger ist ohne Familienuntersuchung *nicht* zulässig. Es ist uns ein noch unveröffentlichter Fall bekannt, bei dem es sich nach kategorischer Aussage um den zweiten, angeblich völlig gesunden homozygoten Pelger-Menschen handeln soll. Diese Aussage wird gemacht, ohne daß auch nur *ein* Familienmitglied untersucht ist. Die Verwendung genetischer Begriffe ist in derartigen Fällen unstatthaft und sollte vermieden werden.

Was ich hiermit über die *Pelger*-Anomalie gesagt habe, gilt aber mutatis mutandis auch für andere Erbmerkmale. Die Erfahrungen, die wir in den letzten 15 Jahren bei Mensch und Tier über das Vorkommen von Phänokopien gesammelt haben, mahnen zur Vorsicht. Freilich möchte ich auch umgekehrt davor warnen, die Häufigkeit der Phänokopien zu *über*schätzen. Wenn z.B. unter dem Eindruck der Beobachtungen von Embryopathien bei Virusinfektion der schwangeren Mutter und der Ergebnisse von Tierversuchen gesagt wird, daß bei der Entstehung der menschlichen Mißbildungen und Mißbildungskrankheiten die Erbanlage neben den peristatischen Faktoren nur eine ganz untergeordnete Rolle spiele, so ist das m.E. mehr eine gefühlsmäßig geäußerte als eine wissenschaftlich fundierte Behauptung. Doch ich kann auf dieses Thema hier nicht näher eingehen, darf aber auf eine demnächst in «Ex-

perientia» erscheinende Abhandlung von mir verweisen, in der ich die Problematik der Phänokopien bei Mensch und Säugetier in ihrer Bedeutung für Humangenetik und Eugenik eingehend behandelt habe.

Schließlich noch ein kurzer Hinweis auf ein weiteres Arbeitsgebiet. Wir haben in den letzten Jahren vergleichende phänogenetische Untersuchungen an einer Reihe erblicher und nichterblicher, letztere durch chemische und physikalische Faktoren induzierter Katarakte des Kaninchens durchgeführt. Mein Mitarbeiter *Ehling* demonstriert die bisherigen Ergebnisse in der Ausstellung dieses Kongresses. Aus seinen Befunden seien hier die an einer einfach-rezessiv erblichen Katarakt erwähnt, die sich in drei Etappen entwickelt: 1. isolierte Trübung der hinteren Linsennaht, 2. Trübung der hinteren Linsenrinde, 3. Totaltrübung der Linse. Nur die Entwicklung der relativ harmlosen Nahtbändchentrübung ist genbedingt, Stadium 2 und 3 sind von exogenen Faktoren abhängig, und es gelang *Ehling* durch Beeinflussung des Wasserhaushaltes des Tieres, die Entwicklung von Stadium 2 und 3 zu unterdrücken. Das eröffnet vielleicht die Möglichkeit einer konservativen Therapie der Katarakt.

In einem zweiten Teil meines Referates wollte ich eigentlich noch einen Ausblick auf zukünftige Aufgaben der vergleichenden und experimentellen Erbpathologie geben. Aber die Zeit ist zu knapp. Ich kann nicht mehr tun, als die Themen wiederholen, die ich schon in meiner im Book of Abstracts des Kongresses erschienenen Zusammenfassung meines Referates genannt habe:

Blutgruppen und genische Inkompatibilität zwischen Mutter und Frucht – genetische Mechanismen der Resistenzentstehung gegenüber Pharmaka – Therapeutika und Erbschädigung – sowie last not least: Atomenergie und Erbgut.

Manche von diesen Problemen sind bereits in Versuchen am Säugetier in Angriff genommen. Ich erinnere nur an die wertvollen Untersuchungen der beiden *Russells* in Oak Ridge an Mäusen zum letztgenannten Problem. Aber es ist doch nur ein Anfang. Die Versuche sind mühsam und schwierig, zeitraubend und kostspielig, da sie, um beweiskräftige Ergebnisse zu bringen, auf breitester Basis durchgeführt werden müssen. Die Arbeiten sollten in internationaler Zusammenarbeit unternommen werden. Die Probleme sind, möchte ich meinen, des Schweißes der Edeln wert.

LITERATUR

Ehling, U.: Untersuchungen zur kausalen Genese erblicher Katarakte beim Kaninchen. Erscheint in Z. Konst.lehre *34*, 1957.

Freye, Hedwig: Anatomische und entwicklungsgeschichtliche Untersuchungen am Skelett normaler und oligodaktyler Mäuse. Wiss. Z. Univ. Halle, Math.-Nat., *3*, 801–824, 1954.

Harm, Helga: Beeinflussung des weißen Blutbildes von Pelger- und Nicht-Pelger-Kaninchen durch Colchicin. Acta haemat. *10*, 95–105, 1953.

Hertwig, Paula: Zwei subletale rezessive Mutationen in der Nachkommenschaft von röntgenbestrahlten Mäusen. Erbarzt *6*, 41–43, 1939.

— Sechs neue Mutationen bei der Hausmaus in ihrer Bedeutung für allgemeine Vererbungsfragen. Z. Konst.lehre *26*, 1–21, 1942.

— Neue Mutationen und Koppelungsgruppen bei der Hausmaus. Z. indukt. Abstamm.- u. Vererb.lehre *80*, 220–246, 1942.

Lüers, Thea, H. Nachtsheim und *G. Petzel:* Phänokopie des Blutbildes des homozygoten Pelgers infolge reaktiver Linksverschiebung bei einem heterozygoten Pelger. Blut *2*, 177–187, 1956.

Michelson, Ann M., E.S. Russell and *P.J. Harman:* Dystrophia muscularis: a hereditary primary myopathy in the house mouse. Proc. Nat. Acad. Sc. *41*, 1079–1084, 1955.

Nachtsheim, H.: Erbpathologie des Stützgewebes der Säugetiere. Handb. d. Erbbiol. d. Menschen *3*, 46–104, 1940.

— Die Mutabilität menschlicher Gene. Caryologia, Suppl. zu *6*, 139–154, 1954.
Mutation und Phänokopie bei Säugetier und Mensch. Ihre theoretische und praktische Bedeutung für Genetik und Eugenik. Erscheint in: Experientia *13*, 1957.

Searle, A.G.: Hereditary "split-hand" in the domestic cat. Ann. Eugen. *17*, 279–282, 1953.

Motulsky, A. G., R. R. Huestis and *R. Anderson:* Acta genet. *6*, 240–245, 1956

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HEREDITARY SPHEROCYTOSIS IN MOUSE AND MAN

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Experimentation on laboratory animals is widely used in medical research and significant information on mechanisms of acquired human disease has been uncovered in this manner. The employment of animals for investigation of hereditary diseases in man has been rather limited. Hemophilia in dogs, *Pelger* abnormality and osteopetrosis in rabbits are a few examples of successfully studied diseases common to both man and animals. Further cooperation between experimental geneticists and medical investigators probably will uncover more areas of common interest as illustrated by the present investigation.

This study represents observations on a new hereditary blood disease in the deer mouse (Peromyscus). Studies to be presented resulted in the conclusion that the condition in the mouse closely resembled hereditary spherocytosis in man.

Hereditary spherocytosis in man has a long history and is known in the older literature as acholuric jaundice or congenital hemolytic anemia. Clinically, affected patients have mild anemia, jaundice and splenomegaly and a high incidence of gallstones. Hematologic investigations reveal red cell spherocytosis reflected by increased osmotic fragility and evidence of severe blood destruction as apparent by shortened red cell survival and high heme pigment output. Severe anemia is usually prevented by increased bone marrow production as manifested by erythroid hyperplasia which is also reflected by the high reticulocyte count. Splenectomy cures these patients by preventing the severe red cell destruction. Family studies indicate that affected patients are heterozygotes.

Against this background we started study of the mutant strain in mice. It had been noted that occasionally newborn mice of the peromyscus colony were jaundiced and pale. Pallor could be correlated with significant anemia. This anemia had essentially disappeared by the tenth day of life.

The blood smears of young and adult affected animals showed spherocytosis, variability in shape and size, and polychromasia reflecting the increased reticulocyte count. Red cell diameters of affected animals were diminished and there was variability of size when compared with the normal as demonstrated by *Price-Jones* curves.

Adult affected animals had no significant anemia. The absence of anemia was explained by the marked bone marrow erythroid hyperplasia which could be recognized by gross inspection of bones or by examination of bone marrow smears. The increased red cell production was most easily detected by elevated reticulocyte counts in the peripheral blood: 14.9% reticulocytes in spherocytic animals as compared with 1.65% in normal animals. If these reticulocyte values were translated to absolute reticulocyte counts, affected animals had eight times the number of reticulocytes than normal animals. This suggests that the affected bone marrow produced eight times more red cells than the normal marrow.

Osmotic fragility tests revealed markedly increased osmotic fragility in spherocytic animals. No electrophoretic hemoglobin abnormality could be demonstrated in affected animals, however a double hemoglobin component was seen in the hemolysates of all classes of animals tested.

The grossly enlarged spleens of the affected animals were much darker in color which reflects the increased red cell pulp content. The increased amount of blood in the splenic pulp was also seen by examination of histologic slides which showed marked red cell congestion in the pulp when compared with normal. Hemochromogen determination showed that spherocytic spleens contained three times as much blood per unit mass and, since they were eight times larger, contained twenty-four times as much blood as the normal spleen.

The role of the spleen in erythrocyte destruction was explored by means of red cell survival time determinations in normal and splenectomized mice. The experiments consisted in tagging the respective mouse red cells with Chromium^{51} and observing the disappearance of the radioactivity. As expected, red cell survival of affected red cells was significantly shorter than normal. A series of red cell survival time determinations were then performed to elucidate the mechanism of hemolysis. Normal cells when injected into the spherocytic animals with large spleens had perfectly

normal red cell survival. These results mean that the enlarged spleens failed to destroy normal cells. Only when spherocytic cells were transfused was there shortening of red cell survival. The shortened survival was strictly a function of the abnormal red cell since spherocytes had also shortened survival in normal recipients. A large spleen was not required for red cell life shortening. In fact, spherocyte survival was shorter in animals with normal spleens than in those with spherocytic spleen. This may be interpreted as the ability of the normal spleen to trap spherocytes more readily than a spleen already previously filled with spherocytes. When the spleen was removed, survival time was normal. In those cases where splenectomy did not result in normal red cell survival, postmortem examination revealed small accessory spleens.

Success of splenectomy was also demonstrated by the marked lowering of reticulocyte counts to 4.7% which reflect lessened need of bone marrow red cell production in response to improvement of red cell survival.*

No difference between normal mice and heterozygotic carriers of the spherocytosis gene could be detected with most of the tests performed. (Hb, Osmotic Fragility, Spleen size, red cell life.) The only detectable difference in reticulocyte counts between normal (1.65%) and heterozygotes (3.6%) was therefore thought to be of questionable biologic significance.

The results of all investigations established the apparent identity of the mouse disease with human hereditary spherocytosis. All hematologic findings were essentially identical. Marked bone marrow overproduction which compensates for the red cell destruction and prevents significant anemia also occurs in man. The histologic findings of the spleen were quite similar. More significantly, red cell survival time determinations in patients have established an identical pattern of splenic destruction. Spherocytes were destroyed by both normal and enlarged spleens. Normal cells survived normally in spite of the enlarged spleen. These studies led to the conclusion that the genetically abnormal cell is trapped by any spleen and destroyed there. Splenectomy removes the trap, but does not basically alter the genetic defect since spherocytosis remains following the operation.

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* The reticulocyte value of 4.7% after splenectomy was not entirely normal and was not due to Bartonellosis flare-up which was controlled by terramycin. Actually the normal red cell life span after splenectomy represents somewhat shortened red cell survival since all cells used for these studies came from actively hemolyzing animals and were young. If such cells would survive normally, the survival graph should be somewhat longer than normal.

The reason why spherocytes are trapped by the spleen is not completely elucidated. However, there is some evidence for the following train of events. Because of the genetically abnormal cytoskeleton, spherocytes are delayed in traversing the splenic pulp. Since extensive concentration of cells occurs in the spleen, there is some reason to believe that glucose depletion may occur. Studies, so far only performed with human spherocytes, suggest a defect of carbohydrate metabolism of the red cell which makes these cells sensitive to glucose lack. Since the red cell membrane needs glucose for proper functioning, membrane "deterioration" occurs which is followed by cell death. Splenectomy removes the selective trap and allows cells to have a normal or almost normal survival in spite of persistence of the genetic defect.

Genetics

The genetic background of murine hereditary spherocytosis was tested by a series of matings. In the breeding experiments, identification was most readily made by observations for pallor and jaundice at birth. This was further checked by appropriate hematologic studies and postmortem examination for splenomegaly. When affected (sp/sp) animals were mated with other affected (sp/sp) animals, all offspring was also affected (sp/sp). When affected animals (sp/sp) were bred with trapped wild normal mice (+/+), no offspring were affected (+/sp). When the F1 generation of the affected and wild matings (+/sp) was back crossed with affected animals (sp/sp), one-half of the young were affected (sp/sp) but one-half were phenotypically normal (+/sp). No sex differences were apparent. It could be concluded that affected animals were homozygotes for the mutant gene. Hereditary spherocytosis in the mouse, therefore, is inherited as an autosomal recessive. As indicated before, criteria of neonatal jaundice and pallor, spleen size and pertinent hematologic studies failed to identify the heterozygote. The penetrance of the syndrome in the mouse appears high since the ratio of affected and normal animals was extremely close to calculated expectations. This is in keeping with the fact that affected animals have a double dose of the gene and that careful examination in regards to multiple criteria is possible.

However, genetic modifiers appear operative if one uses the severity of neonatal jaundice as a criterion of severity of disease. It was noted that some affected animals were more severely jaundiced than others. When the young "very yellow" animal were segregated and mated with each other, approximately two-thirds of the offspring were also intensely

jaundiced at birth. If the "very yellow" affected animals were mated with a "yellow" partner, only twenty per cent of the young were "very yellow". Matings of the average type of yellow mouse with each other only resulted in very few "very yellow" offspring. It should be understood that all offspring of these matings were affected with spherocytosis. No definite genetic interpretation of these experiments is possible yet. However, the role of genetic factors in determining severity of the syndrome cannot be doubted. Critical studies are in progress to test whether severe jaundice in these animals represents a more severe hemolytic syndrome or is caused by lessened capacity of the liver to remove bilirubin.

Data are available to suggest that different genetic modifications of human hereditary spherocytosis also occur. Thus, we noticed only partial response to splenectomy in three members of one family. This occurred in spite of careful search for accessory spleens. Biochemical studies of red cells in this family suggested a more severe red cell metabolic defect than found in other kindreds. *Prankerd* and his co-workers noted failure of the red cell metabolic defect to respond to adenosine in some of their kindreds and not in others. More detailed studies on these variants in man are required.

In human hereditary spherocytosis affected patients usually have one affected parent and 50% of the offspring have hereditary spherocytosis. The condition is inherited as a dominant and affected patients are heterozygotes in contrast to affected mice which are homozygotes. Homozygosity for hereditary spherocytosis in man is not definitely known. Although the suggestion has been made that homozygosity may be lethal, the presence of thirteen affected patients in a published French sibship and the relative vigor of our affected homozygous mice suggests that the double dose of the gene may not be as serious as believed. Inheritance of clinically identical disease either as a recessive or dominant is not too unusual. Even in man clinically indistinguishable syndromes may be transmitted as either recessives or dominants, such as in hereditary ataxia.

Studies of human pedigrees have occasionally uncovered unaffected parents. Reduced penetrance and the carrier state for the spherocytosis gene have been invoked to explain such findings. The presence or absence of anemia cannot be used as an index of the presence or absence of hereditary spherocytosis since the bone marrow varies in its compensation in response to red cell destruction. Non-anemic patients, just as non-anemic mice, may have severe blood destruction which may lead to crises as well

as a high incidence of gallstones. Clinically, splenectomy is indicated when blood destruction is significantly increased regardless whether anemia is present or not.

The employment of increasingly refined methods for the detection of hereditary spherocytosis is increasing the apparent penetrance of this gene in man. Such tests are the twenty-four hour incubated osmotic fragility test at 37 degrees as metabolic stress on the defective erythrocyte and the determination of red cell survival. *Newton* recently demonstrated red cell life decrease of one-half to one-fourth of normal in apparently unaffected parents of children with hereditary spherocytosis. The effects of this mild reduction in red cell life would be very difficult to detect in any other manner. Such studies illustrate the fluid nature of the penetrance concept since better detection of genetic action by sensitive techniques may uncover a large number of affected patients.

Little is known about the exact incidence and population dynamics of hereditary spherocytosis in man. Studies on the mouse colony failed to show any significant difference in litter size among the three different types of females, thus ruling out any lethal effect on the offspring. Although we do not have quantitative data on fertility, observations on the many matings carried out failed to suggest any significant reproductive disadvantage in the spherocytic group since matings could be carried out quite easily.

In man, the condition appears more common among whites than among Negroes. Human studies agree on a shortage of affected sibling patients. This may be due to the observed high miscarriage rate and infant mortality as well as due to failure of detecting hereditary spherocytosis in midly affected patients as pointed out above. The high miscarriage rate may be compensated by the increased number of offspring among affected females as noted by Race. These factors, as well as the low mortality, the good results of splenectomy and a steady mutation may be expected to lead to increased frequency of the condition in man.

Conclusions

A new hereditary hemolytic syndrome in the deer mouse was shown to be the clinical and hematologic counterpart of hereditary spherocytosis in man. This animal strain provides a convenient experimental tool for a variety of further studies on hereditary spherocytosis and hereditary hemolytic disease in general. We hope to have shown with this approach that simultaneous animal and clinical investigations in hereditary disease may be used to obtain information of both medical and genetic interest.

Degenhardt, K.-H.: Acta genet. 6, 246–252, 1956

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PHASENSPEZIFITÄT O_2-MANGEL INDUZIERTER WIRBELSÄULENMISSBILDUNGEN BEI KANINCHEN[1]

Von K.-H. DEGENHARDT

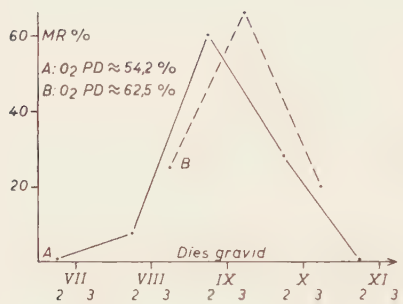
Um die Jahrhundertwende gab *Wilhelm Roux* Anregung zu einer kausalen Analyse der Formbildung und Entwicklung tierischer Organismen und begründete damit eine ganz neue biologische Disziplin, die heute mehr denn je im Brennpunkt der naturwissenschaftlichen Forschung steht. Namhafte Forscher der verschiedensten Länder haben inzwischen fundamentale Erkenntnisse auf dem Gebiete der Entwicklungsphysiologie und Pathologie vermittelt und wesentliche Einblicke in die kausalen Zusammenhänge organischer Gestaltungen gegeben, jedoch brachte jeder weitere Erkenntnisschritt neue und schwerer zu lösende Probleme. Hier knüpfen eigene tierexperimentelle Untersuchungen an mit dem Ziel, speziellen Fragen der embryonalen Teratogenese nachzuspüren und durch vergleichende Untersuchungen den Prinzipien menschlicher Teratologie näherzukommen. Als Vorbild dienten die an verschiedenen Forschungszentren von *Büchner*, *Ingalls*, *Werthemann*, neuerdings auch von russischen und japanischen Forschern an Hühnchen-, Mäuse- und Rattenembryonen durch Sauerstoffmangel erzielten mannigfachen Entwicklungsstörungen. Es ist uns inzwischen gelungen, durch kurzfristigen Sauerstoffmangel bei trächtigen Kaninchen die Embryonalentwicklung phasenspezifisch zu stören und spezielle Entwicklungsbahnen bzw. deren Ak-

[1] Herrn Prof. Dr. *Frh. v. Verschuer* zum 60. Geburtstag. Mit freundlicher Unterstützung der deutschen Forschungsgemeinschaft.

tions- und Reaktionssystem in ihren Induktions- und Differenzierungsleistungen zur Entgleisung zu bringen. Zur Methodik der Versuche sei kurz folgendes bemerkt: Die Muttertiere wurden einmalig in der Zeit zwischen dem 7. 15. Tag der Gravidität für 3 7 Stunden einem Sauerstoffmangel von durchschnittlich 50 mm Hg O_2-Partialdruck exponiert. Wir warteten die Wurfergebnisse ab, begutachteten die äußere Form der Jungtiere, prüften das Skelettsystem im Röntgenbild und obduzierten die überwiegende Zahl der Jungtiere zur Erfassung fehlerhafter innerer Organanlagen. Wir erzielten folgende Ergebnisse: 157 Muttertiere warfen nach O_2-Mangel-Exposition insgesamt 707 Jungtiere, von denen 225 = 32% Entwicklungsstörungen verschiedenster Organbezirke aufwiesen. Zahlenmäßig standen Wirbelsäulenfehlbildungen aller Variationsgrade weitaus im Vordergrund: wir beobachteten diese ausschließlich nach O_2-Mangel-Exposition dies graviditatis VIII X, wobei der 9. Schwangerschaftstag hinsichtlich der Störungsempfindlichkeit axialer Gradienten besonders hervortrat. In parallel laufenden, ausgedehnten Kontrollreihen mit dem gleichen Tiermaterial wurden nach unbeeinflußter Gravidität Mißbildungen dieser Art niemals vorgefunden. Die zusammen mit *Kladetzky* im anatomischen Institut der Universität Köln geführten embryohistologischen Untersuchungen ergaben, daß die fehlerhafte Induktion der Wirbelblasteme in unmittelbarem Zusammenhang steht mit Chordazellschädigungen und dadurch bedingten sekundären Chordaverlagerungen. Es handelt sich bei den durch O_2-Mangel induzierten Wirbelsäulenfehlbildungen demnach um echte Phänokopien der von *Töndury-Theiler* bei Mäusen beobachteten, erblich bedingten Entwicklungsstörungen des Achsenskeletts. Vergleichende morphologische Studien der Wirbelfehlbildungen bei Kaninchen mit entsprechenden menschlichen Fehlbildungen zeigten selbst in feineren pathologischen Strukturelementen hinsichtlich Spezifität, Extensität und lokaler Prädilektion weitgehende Übereinstimmungen, so daß Rückschlüsse auch für die kausale und formale Genese menschlicher Wirbelsäulenmißbildungen gerechtfertigt erscheinen.

Die Analyse der unter bestimmt abgeänderten Versuchsbedingungen nach O_2-Mangel-Exposition erzielten Wurfergebnisse ergab im Hinblick auf die Manifestation axialer Entwicklungsstörungen fein abgestufte, regionalspezifische Wirkungen des exogenen Agens, die sich sowohl auf die Höhe der Mißbildungsraten als auch auf die Lokalisation der Wirbelfehlbildungen im kranio-kaudalen Entwicklungsgefälle beziehen.

Hierzu einige Abbildungen:


60
40
20
MR %
A: O2 PD ≈ 54,2 %
B: O2 PD ≈ 62,5 %
B
A
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VII
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X
XI
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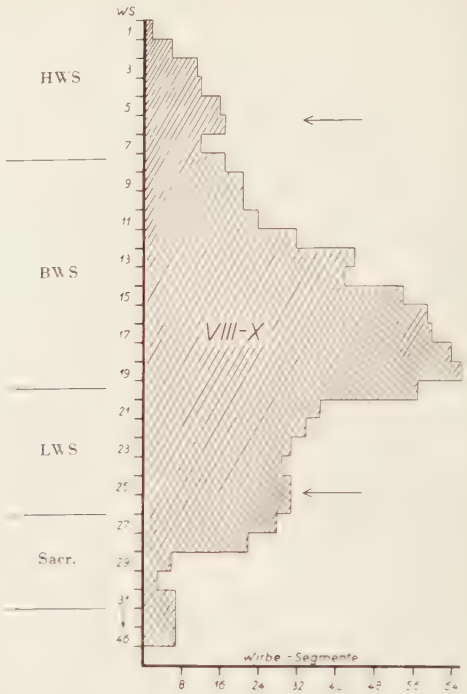
WS
HWS
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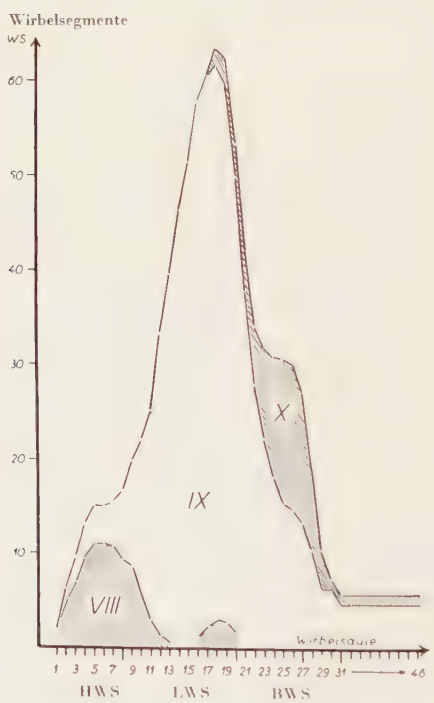
Sacr.
VIII-X
Wirbe - Segmente

Abb. 1


Wirbelsegmente
WS
60
50
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HWS
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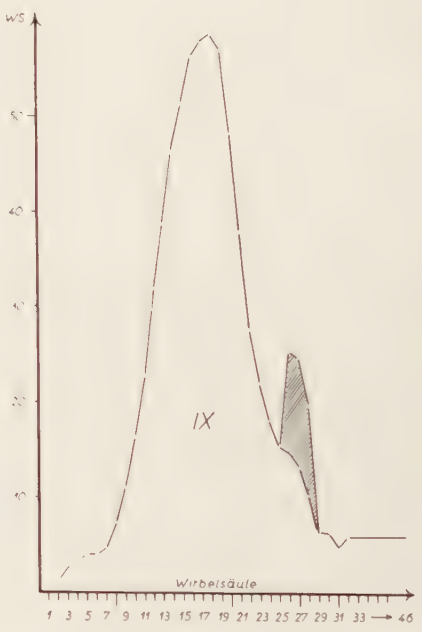
BWS


WS
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Wirbelsäule
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 46

Abb. 3

Abb. 1 zeigt die relativ eng begrenzte, O_2-Mangel empfindliche sensible Entwicklungsphase axialer Organanlagen. Zur Kennzeichnung der Expositionszeiten teilten wir die Schwangerschaftstage in 3 Achtstundenphasen. Die Versuchsreihen der Jahre 1953 54 betrafen regelmäßig die 2. Achtstundenphase, die Versuchsreihen der Jahre 1955 56 vornehmlich die 3. Phase. Die graphische Darstellung der Mißbildungsraten zeigt am 8. Schwangerschaftstag nach geringer zeitlicher Verschiebung der O_2-Mangel-Exposition einen ziemlich steilen Anstieg der Kurve. Das Maximum der Störungsempfindlichkeit liegt eindeutig in der 3. Phase des 9. Schwangerschaftstages: diese Sensibilität fällt zum 11. Tag hin wiederum steil ab.

Abb. 2 demonstriert die Häufigkeit, mit der einzelne Segmente der Wirbelsäule durch O_2-Mangeleinfluß betroffen sind; es zeigt sich eine charakteristische Bevorzugung der unteren Brustwirbelsegmente. Der graphischen Darstellung liegen insgesamt 811 mißgebildete Wirbelsegmente bei 193 Jungtieren zugrunde.

Abb. 3 zeigt die Häufigkeit einzelner mißgebildeter Wirbelsegmente getrennt nach den Expositionszeiten der Muttertiere im O_2-Mangel. Die mit Hilfe des mathematischen Mittels ausgeglichenen Kurven beweisen

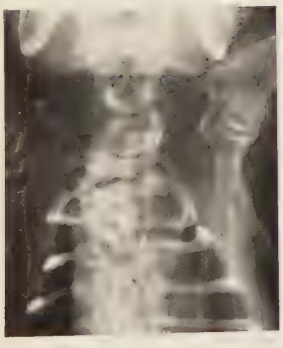

Abb. 5

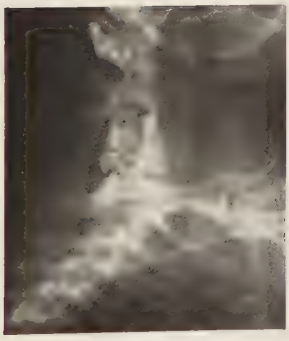

Abb. 6

eine streng phasenspezifische Lokalisation der Wirbelfehlbildungen: Nach O_2-Mangel dies graviditatis VIII findet sich der Manifestationsschwerpunkt in der unteren Halswirbelsäule, am folgenden Tag im Bereich der unteren Brustwirbelsäule und am 10. Tag der Gravidität eindeutig im Bereich der unteren Lendenwirbelsäule.

Abb. 4 hebt den 9. Tag der Gravidität hinsichtlich der Häufigkeit betroffener Wirbelsegmente und der Extensität der axialen Störungsbereiche deutlich hervor. Häufigkeit und Extensität der Fehlbildungen erwiesen sich abhängig von der Intensität des O_2-Mangel-Einflusses: Je größer die Intensität, um so höher lag die Manifestationsrate und um so ausgedehnter war in der Regel der Störungsbereich fehlgebildeter Wirbelsegmente. Relativ häufig traten lumbosakrale Übergangswirbel in Kombination mit Fehlbildungen der Brustwirbelsäule in Erscheinung; die Manifestationsrate dieser charakteristischen Wirbelsäulenvarietät betrug nach O_2-Mangel dies graviditatis IX, 3. Phase 24% gegenüber 2% in den normalen Kontrollreihen. Demnach begünstigt O_2-Mangel die Manifestation lumbosakraler Übergangswirbel auf dem Wege einer stärkeren Seitendifferenz segmentaler Verschiebungen der unteren Extremitätenanlagen. Der in den absteigenden Schenkel der Kurve eingezeichnete schraffierte Keil kennzeichnet den Anteil kombinierter Übergangswirbel.

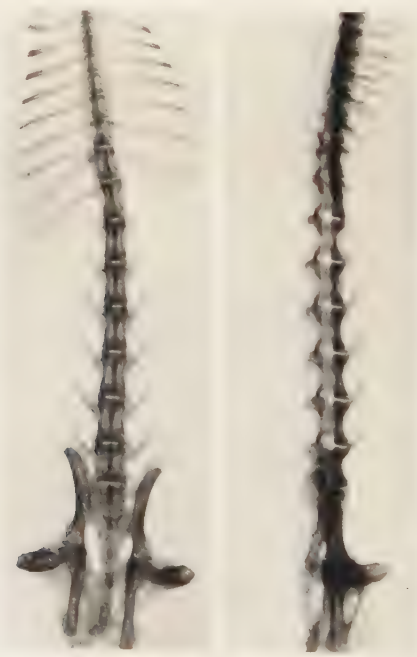

Abb. 7

Nun seien einige charakteristische durch O_2-Mangel induzierte Wirbelsäulenfehlbildungen bei Kaninchen kurz betrachtet. Jede Wirbelsäulenregion zeigt abgesehen von der phasenspezifischen Lokalisation der Fehlbildungen bestimmte typische Gestaltungsphänomene. In diesem Zusammenhang verweise ich auf die Skelettdemonstrationen in der wissenschaftlichen Ausstellung.

Abb. 5 zeigt ein Klippel-Feil-Syndrom mit Spalt- und Blockbildungen der unteren Halswirbelsäule mit typischer Kurzhalsbildung. O_2-Mangel dies graviditatis $VIII_2$.

Abb. 6 zeigt multiple Keil- und Blockbildungen in dem am häufigsten betroffenen Abschnitt der unteren Brustwirbelsäule nach O_2-Mangel dies graviditatis IX_2.

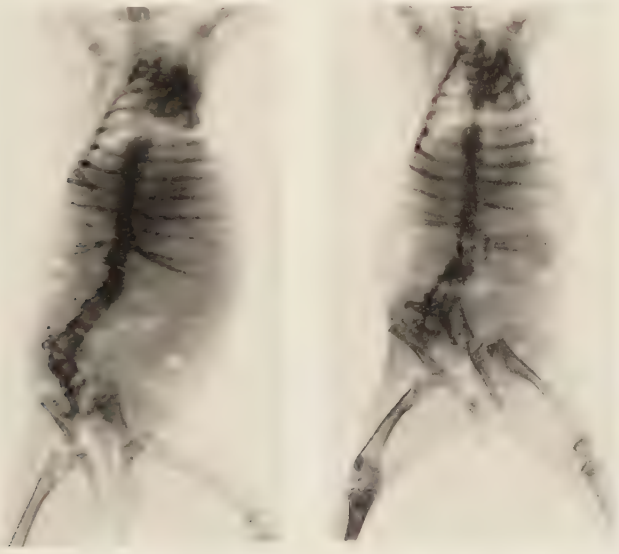

Abb. 8

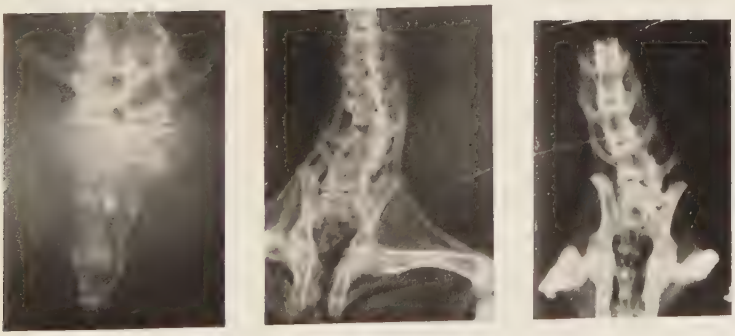

Abb. 9

Abb. 7 zeigt eine häufig beobachtete Kombination fehlgebildeter Brustwirbel-Segmente mit einem lumbosakralen Übergangswirbel, der Beckenschiefstand bedingt.

Abb. 8 zeigt 2 Geschwister eines Wurfes mit schweren Fehlbildungen der unteren Brustwirbelsäule und des gesamten kaudalen Wirbelsäulen-Abschnittes; bei dem einen Tier ist eine exzessive Schwanzhypoplasie erkennbar. O_2-Mangel dies graviditatis IX_2.

Abb. 9 zeigt verschiedene lumbosakrale Wirbelsäulenfehlbildungen nach O_2-Mangel dies graviditatis X_2. Wir beobachteten teils isolierte, häufiger aber kombinierte Wirbelsäulendefekte mit nachfolgender starker Skoliosebildung.

Die beschriebenen Wirbelsäulenfehlbildungen waren in etwa 9% der Fälle mit typischen Fehlbildungen anderer Organsysteme korreliert.

Vetukhiv, M.: Acta genet. *6*, 252–254, 1956

Department of Zoology, Columbia University, New York City, U.S.A.

THE LONGEVITY OF HYBRIDS BETWEEN LOCAL POPULATIONS OF DROSOPHILA PSEUDOOBSCURA

By M. VETUKHIV

Studies on Drosophila populations have elucidated biological regularities which may be important for the human species. Genetic structures that influence longevity in the population are of great importance in evolutionary adaptation. Due to the increasing amount of interlocal crosses between populations, the problem of superior adaptive values of heterozygotes in the F_1, and of the behaviour of F_2 and following generations, in mixed human as well as in animal and plant populations are worth serious consideration.

In earlier experiments *Pearl* [1928], working with Drosophila melanogaster, found that hybrid vigor was lost in F_2. Later *Heuts* [1948]

found in Drosophila pseudoobscura that intermediacy and superiority of F_1 hybrids in longevity depended on the temperature under which the experiments were performed. *Wallace* [1948] showed that the longevity of heterozygotes at 25 C approached that of the superior homozygote. *Comfort* [1953] prepared life tables for successive generations stating that the increase in life span was probably due to a difference in culture conditions. *Jean M. Clarke* and *J. Maynard Smith* [1955] obtained data on the superior longevity of F_1 hybrids of Drosophila subobscura as shown by survival curves and measurement of force of mortality.

The present paper concerns longevity in local populations of Drosophila pseudoobscura and in their F_1 and F_2 hybrids. The material for the experiments consisted of strains derived from wild flies collected at Western United States. All strains were homozygous for the Arrowhead gene arrangement in the third chromosome. Artificial populations were obtained by crossing the strains from each locality. F_1 and F_2 populations were produced from matings between members of the same local populations and by crossing members of different populations.

25 females and 25 males from each population were put in regular bottles with regular food. Four bottles were made for each cross—two replicates and two reciprocal crosses. The flies were kept at temperatures of 16° and 25°C. Every five days bottles kept at 25°, and every seven days those kept at 16° C. were changed and the dead males and females counted. Thus the length of the adult life was determined.

In the means, as well as in the medians, the F_1 are either superior when compared with both parental populations, or are intermediate with approach to the longer-lived parent strain. Probably the medians give the best single comparison. In the F_1 the medians are superior to both parental populations in 7 cases out of 10 at 25° C and in 10 cases out of 10 at 16°C. Three cases were superior to one of the parents at 25°C. For most of the above mentioned cases the differences are statistically significant.

The superiority of F_1 disappears in F_2. Moreover, in many cases the F_2, when compared in median length of life, turns out to be lower not only than F_1, but also than both parents. In 12 cases out of 20 at 25° C and in 10 cases out of 20 at 16°C longevity of F_2 flies were lower than both parents. In 5 cases out of 20 the longevity was lower than one of the parents at 25° C. and correspondingly in 2 cases out of 20 at 16° C. The differences are partly statistically significant.

It may be mentioned that the superiority of the F_1 was more clearly pronounced at 16° C than at 25 C. The breakdown in the F_2 in general

is not so strikingly apparent as the superiority in the F_1, but at 25° it is more evident that at 16° C, so that again the hybrid is relatively more affected by the high temperature than are the parental strains. In general the males are weaker, the females show higher longevity.

In conclusion it may be stated that in longevity F_1 crosses are mostly superior to their parental populations: this is the phenomenon of heterosis. This superiority disappears in F_2; some F_2 crosses are inferior even to the parental populations. This is the phenomenon of hybrid breakdown in interpopulation crosses.

As a result of my previous experiments on the viability it was found that different species of Drosophila give different results as to the heterosis in F_1 and the breakdown in F_2. We do not know what results Homo sapiens will show with respect to heterosis and breakdown in interpopulation crosses, but the results obtained in these experiments with Drosophila point towards desirability of such studies on human populations as well, taking into account, of course, all the peculiarities that are connected with the unique qualities of man.

Sachs, L., M. Danon, M. Feldman and *D. M. Serr:* Acta genet. *6*, 254-255, 1956

Weizmann Institute of Science, Rehovoth, and Rothschild-Hadassah University Hospital, Jerusalem, Israel

THE PRENATAL DIAGNOSIS OF HUMAN ABNORMALITIES

By L. SACHS, M. DANON, M. FELDMAN and D. M. SERR

It has been found that amniotic fluid cells can be used for the prenatal diagnosis of sex and of blood group antigens. The examination of amniotic fluid, which can be safely obtained from the twelfth week of pregnancy, has shown that in all cases investigated from this time onwards there have been a sufficient number of cells for both types of prenatal determination, although many of the cells were degenerating.

The diagnosis of sex is carried out by determining the percentage of non-degenerating nuclei with chromocenters located at the nuclear membrane. The diagnosis of foetal blood group antigens, which has so far been applied to ABO, is based on the existence of a specific mixed agglutination between erythrocytes and amniotic fluid cells coated with the appropiate antiserum.

An early prenatal diagnosis of sex can be of medical value in cases of sexlinked abnormalities, and as an example a new pedigree of a family with sex-linked muscular dystrophy was presented. The prenatal determination of foetal antigens can be of value for an early diagnosis of incompatibility in cases where the father is heterozygous, as well as for the detection of hereditary abnormalities that are genetically linked to an antigen. The medical implications of these possibilities were discussed.

Danon, M. and L. Sachs: Acta genet. *6*, 255–256, 1956

Weizmann Institute of Science, Rehovoth, Israel

THE SEX CHROMOSOMES IN HUMAN INTERSEXES

By M. DANON and L. SACHS

The sex chromosomes have been studied in different types of human intersexes on the basis of an analysis of chromocenters. Special attention has been paid to possible cases of abnormal sex chromosome constitution. In a hereditary type of intersex associated with external female appearance, primary amenorrhea, absence of the uterus and of pubic and axillary hair, and the presence of a testis (testicular feminisation syndrome), the data obtained from the study of pedigrees can be explained in that the intersexes are either XY or XXY. An analysis of chromocenters suggests that they are XXY. An examination of chromocenters in patients with *Turner*'s syndrome has shown that these cases can be either

XX, XY or XO, or a mosaic with different sex chromosome constitutions. A general scheme was presented that can explain the origin of the different types of human intersexes.

Discussion:

Dr. *P. C. Koller* (London): I would like to draw the attention of Dr. *Sachs* that he may be able to add another criteria for determining the sex-chromosome constitution in individuals with sex-disorder. *Poloni* and his coll. in England made use of a sex-linked gene (colour-blindness) to determine the presence or absence of Y chromosome in ovarian agenesis. The genetic criteria would be another additional evidence to disclose the XX or XY constitution in Dr. *Sachs*' so excellently analysed cases.

Riis, P., F. Fuchs, S. G. Johnsen, J. Mosbech and *C. E. Pilgaard:* Acta genet. 6, 256–260, 1956

The Copenhagen County Hospital, Hellerup
The University Hospital (Rigshospitalet), Copenhagen
and Rudolf Berghs Hospital, Copenhagen, Denmark.

CYTOLOGICAL SEX DETERMINATION IN DISORDERS OF SEXUAL DEVELOPMENT

By P. RIIS, F. FUCHS, S. G. JOHNSEN, J. MOSBECH and C. E. PILGAARD

A number of patients with various congenital errors of sexual development have been examined with regard to the so-called cellular sex, discovered by *Barr* and his associates [1, 2]. These workers found that the majority of cell nuclei from females contains an easily detectable chromatin condensation with a characteristic localization.

Methods

The original methods for cellular sex determination were based upon skin or mucous membrane biopsies. We, as well as other workers, have found, however, that dependable results can be obtained with blood

smears and mucous membrane smears. All our cases have been examined by oral and urogenital smears, and, in the majority of cases, also by blood smears. We have found urogenital smears superior to oral smears, and oral smears superior to blood smears. We shall therefore give a short description of the method based upon the use of urogenital smears.

During the development of a method for antenatal sex determination of the foetus, which has been described by two of us [5], we found that the amniotic fluid cells exhibited easily detectable sex chromatin in cases of a female foetus.

To find the origin of these cells we carried out comparative studies of the nuclear morphology of amniotic fluid cells and epithelial cells from the mucous membranes of children and adults. We hereby found that those amniotic fluid cells which gave the clearest picture of sex chomatin most likely were of vulvo-vaginal origin. This led to a study of the possibility of using smears from the external genitals of both sexes for postnatal sex determination. Samples were collected from females by touching the mucous membranes in the vulva in females and the distal part of the urethra in males with a saline-moistened cotton swab. The smears were made on egg-albumen-coated slides and prepared with a slight modification of *Papanicolaou*'s method, including staining with chresyl-echt-violet. A total of 67 persons were examined, 36 females and 31 males, ranging in age from a few weeks to 78 years. The sex of the examined persons was unknown to the investigator until the histological sex diagnosis had been made. In none of the cases a discrepancy between cellular and phenotypic sex occurred [11].

This method was developed independently by the Dutch workers *Carpentier*, *Stolte* and *Visschers*, who confirm its superiority to the methods previously described [4].

In connection with this investigation we tried whether a routine cellular sex diagnosis could be made on desquamated epithelial cells from a single portion of urine. A total of another 86 females and males were examined. In all females enough cellular material was found in the urinary sediment to establish a correct sex diagnosis. Unfortunately this was not the case with some of the male samples. The urine method, therefore, cannot be recommended for a screening purpose.

Material

Cellular sex determination was carried out in a series of ten patients with *Turner*'s syndrome and related forms of "gonadal dysgenesis". These patients were seen at the University Hospital of Copenhagen [7]. In

accordance with the results of other workers [6] we found the following sex distribution: 8 out of the 10 patients showed male, or with a synonymous and better term, chromatin-negative nuclei.

A number of patients with severe male hypogonadism were also studied. Until now a total of 21 patients have been examined, representing most forms of severe male primary hypogonadism. The series included 5 cases of *Klinefelter*'s syndrome [9]. These are patients characterized by gynaecomastia, small testes with aspermatogenesis, normal or reduced function of the *Leydig* cells and mostly increased excretion of the follicle-stimulating hormone. The general appearance of these patients is male, with an almost normal penis and a normal or small scrotum (figs. 1 and 2).

Fig. 1. A 25 year old patient with *Klinefelter*'s syndrome. Note the well-developed gynaecomastia.

Fig. 2. The external genitals of the same patient.

No signs of rudimentary female internal or external genitals are present. In testicular biopsies hyalinisation of the seminiferous tubules is seen. The breast tissue shows marked proliferation of the periductal connective tissue.

Sex determination showed the cellular sex to be male in all cases of hypogonadism except in *Klinefelter*'s syndrome. The five patients with *Klinefelter*'s syndrome were shown to have chromatin-positive ("female") nuclei in epithelial smears and blood smears [12]. This is in accordance with recent reports from Canada [10] and South Africa [8].

These new findings have several interesting aspects, including endocrinological, psychological and medico-legal. The theoretical aspects in relation to human genetics deserve special mention. When it was first shown that the majority of cases with *Turner*'s syndrome had chromatin-negative ("male") nuclei, some of the theories concerning human sexual development apparently had to be revised [6]. Experimental investigations of laboratory animals seemed to show that the lack of testicular tissue in a developing male organism would convert the sexual differentiation in feminine direction. The reported finding in patients with *Klinefelter*'s syndrome would seem to indicate that this syndrome represents a mirror-image of *Turner*'s syndrome. We lack, however, supporting experimental evidence that a genetic female organism can develop into a phenotypic male. *Klinefelter*'s syndrome therefore might be explained by other mechanisms. Theoretically two factors could be responsible for the sexual conversion: a local strong masculinizing "inductor", of cellular character, or a hormonal factor, for instance adrenal of origin. From the work on animal embryos we know that the local presence of testicular tissue is necessary for the development of the normal male external and internal genital ducts, at least in certain animals. On the other hand, no primary hormonal disturbances have until now been described in patients with *Klinefelter*'s syndrome.

The reported discrepancy between cellular and probably genetic sex on one side and sexual fænotype on the other in cases of *Klinefelter*'s syndrome yields new information about the complexity of factors influencing human sexual development. It seems that under certain conditions unknown factors are able to convert the sexual development further in the direction of the opposite sex than it was realized before.

Whether these factors are genetically determined or of exogenous nature is not known. In relation to this it may be mentioned that a case of *Turner*'s syndrome and a case of *Klinefelter*'s syndrome have recently been described among siblings [3]. Patients with such severe disturbances

in early embryonic development are non-fertile on account of the accompanying aspermatogenesis or anovulation. This complicates genetic studies, but sibling-studies should certainly be carried out.

REFERENCES

1. *Barr, M. L.* and *E. G. Bertram:* Nature *163*, 676, 1949.
2. *Barr, M. L.:* Modern Trends in Obstetrics and Gynaecology II: p. 126. Butterworth & Co., London 1955.
3. *Bassø, H. H.:* Nord. Med. *56*, 969, 1956.
4. *Carpentier, P. S., L. A. M. Stolte* and *G. P. Visschers:* J. clin. Endocrinol. *16*, 155, 1956.
5. *Fuchs, F., P. Riis:* Nature *177*, 330, 1956.
6. *Grumbach, M. M., J. J. v. Wyk* and *L. Wilkins:* J. clin. Endocrinol. *15*, 1161, 1955.
7. *Hammen, Rich.:* Meeting at the "Society of Gynaecology and Obstetrics", Copenhagen, April 20, 1956.
8. *Jackson, W. P. U., B. G. Shapiro, C. J. Uys* and *R. Hoffenberg:* Lancet *1*, 969, 1956.
9. *Klinefelter, H. F.*, Jr., *E. C. Reifenstein* Jr. and *F. Albright*: J. clin. Endocrinol. *2*, 615, 1942.
10. *Plunkett, E. R.* and *M. L. Barr:* Anat. Rec. *124*, 348, 1956.
11. *Riis, P.* and *C. E. Pilgaard:* Acta path. microbiol. scand. In press.
12. *Riis, P., S. G. Johnsen* and *J. Mosbech:* Lancet *1956 I*, 962.

Discussion:

Dr. *W. Lenz* (Hamburg): Eine von *Reifenstein* beschriebene Familie mit *Klinefelter*-Syndrom zeigt rezessiv-geschlechtsgebundene Vererbung. Ist das mit der XX-Konstitution des *Klinefelter*-Syndroms vereinbar? Kann es sich um ein abnormes X-Chromosom neben einem Y-Chromosom handeln?

Fuchs, F., E. Freiesleben, E. E. Knudsen and *P. Riis:* Acta genet. *6*, 261–263, 1956

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ANTENATAL DETECTION OF HEREDITARY DISEASES

By F. FUCHS, E. FREIESLEBEN, E. E. KNUDSEN and P. RIIS

Until now, antenatal prediction of hereditary disorders has had to be based upon statistical probabilities. Recent discoveries indicate, however, that in the future it will be possible to diagnose certain hereditary diseases by direct examination of foetal cells, obtained without interruption of pregnancy.

The determination of the foetal sex by examination of the desquamated cells in the amniotic fluid for the specific sex chromatin, discovered by *Barr* and his associates [1], has been described by two of us [2]. Independent development of the method simultaneously in various parts of the world has amply confirmed its reliability [3, 4, 5, 6], and it needs no further discussion here.

If the foetal blood-group can be determined from amniotic fluid constituents as early as the sex, that is, from the fourth month of pregnancy, and amniotic fluid can be with drawn at this stage without disturbing the pregnancy—which we believe but so far have only one case to support—then it should be possible to diagnose both sex-linked and blood-group-linked hereditary diseases at a stage where pregnancy can be safely interrupted [7].

No simple and reliable method for determination of the blood-group from tissue cells was available, until *Coombs* and his coworkers [8] recently described a method by which A and B antigens could be demonstrated on human epidermal cells by mixed agglutination. In our hands, this method has made it possible to detect A and B antigens in the cells of amniotic

fluid, all of which of course are of foetal origin. Since this opens new possibilities, the method will be described briefly [9].

For our studies amniotic fluid was obtained by insertion of a catheter into the amniotic cavity from below in cases where labour was to be induced, and by transabdominal puncture in a case of hydramnios. A few milliliters was centrifuged, and the vernix and the supernatant were removed. The cellular sediment was washed and then incubated with anti-A and anti-B immune sera, respectively. The samples were then centrifuged and the sediments washed again. One per cent suspensions of washed erythrocytes of group A, B and O were mixed with the cell suspensions in the following way:

1. Amniotic cells incubated with anti-A+A erythrocytes,
2. Amniotic cells incubated with anti-B+B erythrocytes,
3. Amniotic cells incubated with anti-A+O erythrocytes,
4. Amniotic cells incubated with anti-B+O erythrocytes.

After centrifugation and resuspension the cell-erythrocyte mixtures were inspected on a slide under cover with an ordinary light microscope or a phase-contrast microscope. Where agglutination occurred, the erythrocytes adhered in large numbers to the surface of the much larger amniotic fluid cells, although these did not all participate in the agglutination. No agglutination between cells alone was observed, and usually not between erythrocytes alone either.

When agglutination occurred in mixture no. 1, a foetal blood-group of A was diagnosed; agglutination in no. 2 was diagnosed as group B, in both as group AB and no agglutination as group O. No agglutination occurred in mixtures nos. 3 and 4, thus providing a control.

So far, we have only studied a limited number of samples and we are trying variations in the procedure to find the simplest and most reliable method. We have had a few failures, but in 20 cases the predicted blood-group has agreed with the group obtained postnatally on cord blood, and we have no doubt that the foetal ABO-group can be diagnosed accurately by this method in the last part of pregnancy. How early in pregnancy it can be done is not known, nor has any attempt been made to determine the Rh or other blood-groups as yet. A knowledge of the foetal Rh-group would be of practical value in clinical obstetrics in cases of Rh-immunization.

The determination of the foetal sex and the foetal blood-group would seem to be of value in preventive eugenics, and it is most likely that examination of the cells and perhaps even of other constituents of the amniotic fluid may disclose other genetic properties of the expected child.

REFERENCES

1. *Moore, K. L., M. A. Graham* and *M. L. Barr:* Surg. Gynec. Obstet. *96*, 641, 1953.
2. *Fuchs, F.* and *P. Riis:* Nature *177*, 330, 1956.
3. *Shettles, L. B.:* Amer. J. Obstet. Gynec. *71*, 834, 1956.
4. *Sachs, L., D. M. Serr* and *M. Danon:* Science *123*, 548, 1956.
5. *Makowski, E. L., K. A. Prem* and *J. H. Kaiser:* Science *123*, 542, 1956.
6. *Dewhurst, C. J.:* Lancet *1956/I*, 471.
7. *Edwards, J. H.:* Lancet *1956/I*, 579.
8. *Coombs, R. R. A., D. Bedford* and *L. M. Rouillard:* Lancet *1956/I*, 461.
9. *Fuchs, F., E. Freiesleben, E. E. Knudsen* and *P. Riis:* Lancet *1956/I*, 996.

Mittwoch, U.: Acta genet. *6*, 263, 1956

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SOME OBSERVATIONS ON THE INCIDENCE OF DRUMSTICKS IN POLYMORPHONUCLEAR NEUTROPHIL LEUCOCYTES OF FEMALES

By U. MITTWOCH

Blood films from mongolian imbeciles and controls of both sexes were examined for number of lobes of the polymorphonuclear neutrophil leucocytes and for the incidence of the drumstick appendage. The material consisted of 40 films and 500 cells were classified in each. No drumsticks were found in any of the males. The incidence in female mongols was about one third of that of the controls. This was associated with a decreased lobe count in the mongols. The incidence of drumsticks was found to increase with increasing segmentation of the nuclei from one to five lobes. The incidence of drumsticks in cells of constant lobe number was lower in mongols than in controls.

Ford, C. E. and *J. L. Hamerton:* Acta genet. *6*, 264–266, 1956

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THE CHROMOSOMES OF MAN

By C. E. FORD and J. L. HAMERTON

For a long time it has been widely believed that the human chromosome number was 48 and that this number applied to both men and women, the controversy of the twenties and thirties regarding the presence or absence of a Y-chromosome being forgotten. However, earlier this year, *Tjio* and *Levan* [1] announced that they had regularly counted 46 chromosomes only in dividing cells of tissue cultures established from 4 human embryos. Although the possibility of a regular loss of 2 chromosomes during embryonic development or culture was almost too fanciful to be considered, they recommended that new counts should be made on preparation from testicular material before the obvious conclusion was drawn. This we have done.

We examined testis tissue from three males, aged 47, 53, and 63 years, respectively. The material was obtained at the Churchill Hospital, Oxford, from fresh operative specimens at the moment of removal from the body. After pre-treatment in hypotonic fluid the specimens were fixed in acetic alcohol and stained by the *Feulgen* procedure. Squash preparations were then made. First spermatocytes at diakinesis or metaphase were the most suitable stages for observation and accurate counts were made on 188 cells. Of these, 174 contained either 23 bivalents, or 22 bivalents plus univalent X and Y. The remaining 14 cells contained 22 bivalents or less, and presumably had been damaged during the making of the preparations with consequent loss of one or more bivalents. No cells contained more than 23 bivalents. There were relatively few spermatogonia in mitosis and most of these were damaged, nevertheless a few clear counts of 46 chromosomes were obtained.

The question arises immediately as to how the discrepancy between the recent counts and the older ones is to be explained. Numerical chromosomal polymorphism of the type known in several orthopteran

species has recently been discovered in a small mammal, the common shrew (*Sorex araneus*) [2, 3]. Although such intra-species variation in number provides a possible explanation, it is made very unlikely by the fact that 7 successive individuals with 46 chromosomes have now been recorded (4 of *Tjio* and *Levan;* 3 reported here). A persistent error seems to be more likely. With the older sectioning technique the chromosomes at metaphase, even in the best cells, were closely crowded and not easily resolved, so that counting was not only tedious but involved a considerable element of subjective interpretation. The present more reliable methods are largely dependent upon devices to disperse the chromosomes widely within the cell, namely, pretreatment with colchicine and/or hypotonic fluid, and squashing. In good preparations counts can now be made both quickly and accurately. Other factors may also have contributed to inaccurate counting. Precocious disjunction of the X-Y bivalent in spermatocytes at first metaphase is not infrequent and results in a count of 24 bodies; while in crowded cells it would not be difficult to mistake a small terminal loop of a large bivalent with several chiasmata for an additional small bivalent. In spermatogonia, several of the longer chromosomes often have greatly elongated centric regions at metaphase; these might be overlooked and each arm counted as a separate chromosome.

The X- and Y-chromosomes were associated terminally in most of the spermatocytes examined at diakinesis or metaphase. The exceptions were the few cells already mentioned in which they were present as univalents, and a single instance in which the association between them could not be fully resolved but which strongly suggested a sub-terminal chiasma. While our observations therefore give no support to the possibility of partial sex-linkage, they are not inconsistent with its occurrence.

Twentythree first spermatocytes in stages from late diplotene to mid-diakinesis were clear enough for accurate counts of chiasmata to be made. Total chiasmata per cell varied from 50 to 63, the range being very similar for each of the three men. The mean was 55.9. On the assumption that each cytological chiasma represents a single genetical cross-over (i.e. 50 centimorgans of map length) an estimate of the total genetic length of human chromosomes may be obtained, namely, 27.9 morgans. The only mammalian species with which a comparison can be made is the house mouse. Estimates of the total map length in the male of this species derived from chiasma counts (*Slizynski* [4]) and by a method utilizing linkage test data (*Carter*) gave values of 19.2 and 16.2 morgans, respectively [5]. It would therefore appear that, genetically, the chromosomes of man are appreciably longer than the chromosomes of the mouse.

A final small point is perhaps worth recording. We have experience of testis preparations from 11 mammalian species other than man (rodents, marsupials, an insectivore, and a primate). In all of these species relatively large groups of first spermatocytes at diakinesis or metaphase are separated by long sections of tubule from which these stages are absent. In our human material, on the other hand, the groups of first spermatocytes at diakinesis or metaphase were very small, but were present in almost every small length of tubule examined. This is in accord with the view that the spermatogenic wave is either greatly reduced or absent in man. It would be of interest to know whether similar conditions occur in younger men.

REFERENCES

1. *Tjio, J. H.* and *A. Levan:* Hereditas *42*, 1, 1956.
2. *Sharman, G. B.:* Nature *177*, 941, 1956.
3. *Ford, C. E., J. L. Hamerton* and *G. B. Sharman:* Unpublished results.
4. *Slizynski, B. M.:* J. Genetics *53*, 597, 1955.
5. *Carter, T. C.:* J. Genetics *53*, 21, 1955.

Discussion:

P. C. Koller (London): Dr. *Ford* mentioned some of the causes which might have been responsible for the mistakes of early cytologists in counting the numbers of chromosomes. By re-investigating some of my preparations, I may add another cause. In the human chromosomes there are several large chromosomes with median centromere, and the two lines of the same chromosome could easily be counted as two separate overlapping rod-shaped chromosomes.

As regards partial sex-linkage, we must admit, that there is no cytological proof except the case mentioned by Dr. *Ford*. The proof of partial sex-linkage must be supplied by the geneticists. If it will be established by genetical data without doubt, then we must reconsider the behaviour of X and Y while they are enclosed within the "vesicle", during prophase of meiosis.

C. E. Ford (Harwell): I agree with Dr. *Sachs* that the evidence in favour of partial sex linkage is now very slender and I understand that those concerned with its investigations are of the same opinion. However, the final word must remain with Geneticists whatever cytologists say.

Schultz-Larsen, J.: Acta genet. 6, 267–271, 1956

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SOME OBSERVATIONS ON THE SUBMICROSCOPIC STRUCTURE OF MAMMALIAN CHROMOSOMES

By J. SCHULTZ-LARSEN

Chromosomes have been intensively investigated by cytologists ever since it was discovered that chromosomes were an obligatory part of the vast majority of plant and animal cell nuclei, and that structural differences in the chromosomes corresponded to variations in genetic factors—thus indicating that the chromosomes determined the hereditary characters.

Using suitable chromosomes, light microscopic studies have shown that the chromosome consists of chromonemas and that these latter undergo a cycle of coiling and uncoiling during division of the cell. In addition, it has been possible to demonstrate a longitudinal differentiation of the chromosome. The question arises whether the microscopically visible thread is a structural unit, or whether there are smaller units that escape observation due to the restricted power of resolution of the light microscope. A further problem is whether chromosomes from other animals than those employed in light microscopic research, which can only be differentiated into chromonemas with difficulty or not at all, may nevertheless prove to be described as comprised of several finer threads once they are observed under microscopes with a greater power of resolution. If they are composed of such threads, how many are there? And is it possible to find a longitudinal differentiation?

If cytology is to continue the extension of our knowledge of basic biological matters side by side with genetics, it is necessary to use the electron microscope in order to describe the submicroscopical structures.

I shall here attempt to elucidate the submicroscopical structure of the mammalian chromosomes, using spermatocytes and spermatogones from mice and humans. It is by no means possible to give an final description of the chromosomes' submicroscopic structure—I shall simply report certain observations in connection with a review of the technical possibilities at our disposal at the present time.

A squash technique suitable for electronmicroscopic use has been described in an earlier paper [2]. As was to be expected, however, the size of the object to be viewed radically restricted the power of resolution of the electron microscope, and but little was achieved that was not already possible by means of light microscopy. Several hundred electronmicrographs gave mostly the impression that this method of preparation injured the cells to such an extent that it was difficult to find a general structure. It was reasonable to assume that the prime cause of the structural variations was to be found in the squash technique's liability to damage the preparations, and that distorsions also occurred during air drying due to surface tension.

Some few micrographs, especially of the late prophase, metaphase and anaphase, nevertheless showed that there was an arrangement of transverse bands. A few pictures of early prophases indicated that the chromosome was spiralled similarly to other chromosomes. It was tempting to presume that the transverse bands were single loops of the densely spiralled chromosomes nearing the metaphase.

In order to avoid the artefacts arising from the surface tension during drying, the preparation technique was so modified that it became possible to use *T. F. Andersson*'s "critical point method" [1]. The squash preparations were made on slide covered with a formvar membrane, then examined under a light microscope, and suitable groups of chromosomes selected. The whole preparation was dehydrated in increasing concentrations of alcohol and put into a strong metal box which was then connected to a cylinder containing carbon dioxide. The alcohol was replaced by liquid carbon dioxide, the temperature raised above carbon dioxide's critical temperature, and the carbon dioxide thus became gaseous without altering the surface tension. The carbon dioxide was then removed and the preparation being dry. A suitable group of chromosomes was then collected on the electron microscope's specimen screen in the manner described in a previous paper [2], and placed in the microscope.

The micrographs show once again that the squash procedure leads to severe artefacts, as it is still difficult to find a common chromosomal structure. However, the procedure confirms that there is a spiral structure

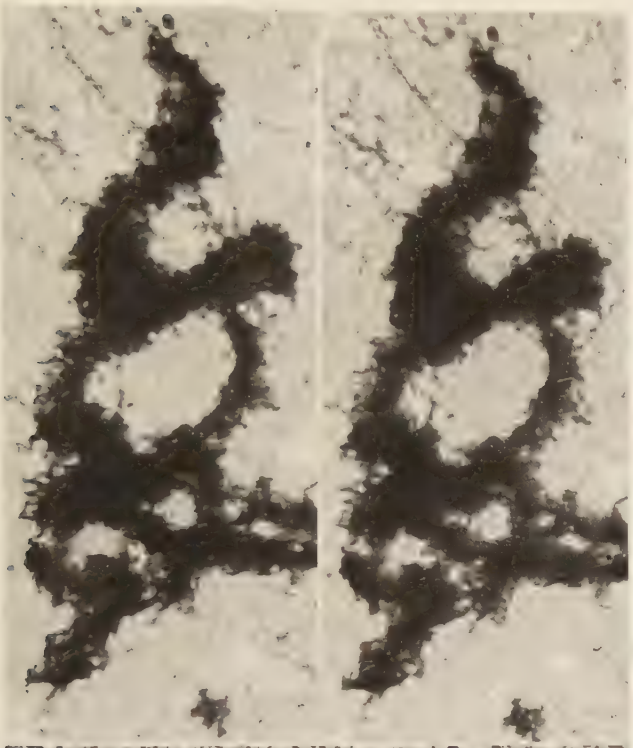

Fig. 1a and *b*. Mouse chromosomes. Stereomicrograph from a squash preparation of chromosomes in early prophase, built up of 1000 Å fibrils, which again consist of 200 Å spiralled fibrils. Magnification: 10,000×.

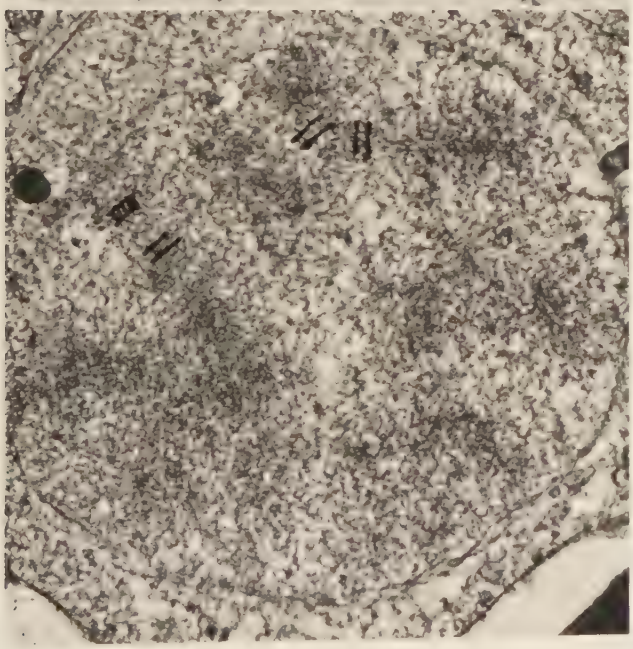

Fig. 2. Mouse chromosomes. 1000 Å sections of spermatocytes with chromosomes in prophase. The embedding material removed and the preparation dried by the "critical point method". In the nucleoplasm one sees fibrils 1000 Å in thickness, which again seem to be composed of 200 Å fibrils. Magnification: 15,000 .

in prophase chromosomes similar to that one finds in a few preparations prepared without using *Andersson*'s "critical point method". The micrographs are clearer, though, and *Andersson*'s drying method means that the chromosomes retain their "natural" three-dimensional arrangement, as they are not pressed down onto the supporting membrane during drying. This makes it possible to take stereoscopic electronmicrographs, which gives a far better impression of the structure of the chromosomes than two-dimensional micrographs.

The restriction of the electron microscope's power of resolution due to the size of the chromosomes can naturally not be avoided by changing the drying technique. The pictures shown hitherto differ only from those obtainable by means of phase-contrast microscopy in their clarity of detail. However, some few preparations which have been loosened in structure during drying and squashing clearly show that the chromosome is composed of fibrils some 1 000 Å thick when photographed stereoscopically. These stereo-electronmicrographs furthermore show that these fibrils are themselves composed of spiralled fibrils approximately 150 200 Å thick (figs. 1a and b).

One may improve the electron microscope's resolution by making ultra-thin sections. Fig. 2 shows a spermatocyte fixed in 1% osmic acid, pH 7.4 in isotonic solution, embedded in metacrylate, and sectioned with a *Spencer* microtome. As the sections are about 1000 Å thick, the metacrylate has been removed prior to investigating them in the electron microscope, and *Andersson*'s "critical point method" has again been used in order to obviate artefacts due to surface tension during drying.

The structures of the contents of the nucleus is simply a fibrillary network without any globular elements whatsoever. In certain areas, assumed to correspond to chromosomes in the prophase, one observes a more directed fibrillary structure of approximately 1000 Å. In a few places it is also possible to see that these fibrils consist of a spiralled fibril about 200 Å thick.

The resolution of the electron microscope is even further improved by employing sections of 200 Å or so. These sections have been obtained by means of a *Sjøstrand* microtome [3]. When sections of this thickness are available, it is no longer necessary to remove the embedding material, and this is a second way in which one may avoid artefacts due to surface tension.

No form of globular elements are observable in these sections, and you will remember that the thicker sections also failed to show such elements. The fibrillary construction observed in the thicker sections is again

present in the 200 Å sections[1]. Generally speaking, the pictures seem quite unsystematic in structure, but more detailed analysis seems to suggest that there is a structure composed of fibrils of several various thicknesses.

Discussion

In conclusion, it must therefore be said that the squash technique is not particularly practical for use in electronmicroscopy. The chromosomes are so severely damaged during this form of preparation, that it is exceedingly difficult to find general structural details, and at the same time the size of the chromosomes restricts the resolution of the electron microscope. Only a very few micrographs show the spiral structure known from other types of chromosomes investigated by means of light microscopy. When the chromosome structure has been loosened up during preparation to some extent, it is nevertheless possible to observe that the coarse spiral loops are made up of fibrils as thin as 200 Å.

Sections likewise manifest fibrillary formations, and it is therefore natural to assume that there is scarcely anything but fibrils in the chromosomes. No form of membrane-like separation of the chromosomes from the surrounding nucleoplasma has been observed in either squash or sectioned preparations.

The observations described here are, however, based on only a few micrographs, selected from several hundreds, and this fully shows how horribly inadequate our present techniques are when we try to describe the structure of the chromosome by means of electronmicroscopy.

Whether the fibrillary elements consists of protein or DNA is an unsolved problem as yet, as it has hitherto been impossible to identify the chemical nature of these structures in the electron microscope.

As the thickness of the fibrils in sections of 200 Å can be observed down to sizes of 200 Å, and as one from a biochemical viewpoint has reached the conclusion that DNA in the chromosomes may be regarded as a large chain molecule, the morphological data at our disposal support the contention that the fundamental structure of the chromosome is fibrillary.

REFERENCES

1. *Anderson, T. F.:* Tr. N. York Acad. Sc. *13/2*, 130, 1951.
2. *Schultz-Larsen, J., M. Hauge* and *F. Carlsen:* Acta path. microbiol. scand. *30*, 306, 1952.
3. *Sjøstrand, F. S.:* Experientia *9*, 114, 1953.

[1] The contrast in the pictures of 100–200 Å sections is so small that it seems quite impossible to get the details in reproductions. Prints will be sent on request.

Leuchtenberger, Cecilie, D. R. Weir, F. Schrader and *R. Leuchtenberger:* Acta genet. *6*, 272–278, 1956

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DECREASED AMOUNTS OF DESOXYRIBOSE NUCLEIC ACID (DNA) IN MALE GERM CELLS AS A POSSIBLE CAUSE OF HUMAN MALE INFERTILITY[1]

CECILIE LEUCHTENBERGER, D. R. WEIR, F. SCHRADER and R. LEUCHTENBERGER

In 1953 *Leuchtenberger* et al. published the first data pertaining to the DNA content of *individual* human spermatozoa. Using *Feulgen* microspectrophotometry which allows the quantitative determination of DNA in individual cells (*Leuchtenberger* [1954]) a remarkably constant haploid amount of DNA was found in the spermatozoa of fertile men while infertile men showed significantly lower DNA values in their spermatozoa.

In the present study which deals with the DNA measurements in nearly 10,000 individual spermatozoa and spermatogenic cells from 25 fertile and 35 infertile men, several pertinent questions were investigated. The fertile group consisted of husbands who had fathered from 1–3 children while the infertile group consisted of husbands from infertile couples who were examined clinically according to the standards of the American Society for the Study of Sterility. None of the wives or husbands showed clinical defects which could be presumed to account for the barren marriage.

Before discussing these problems a short review of the basic findings seems indicated. In fig. 1, DNA values in spermatozoa of 21 fertile males and 21 infertile males are presented. It is evident that the 21 fertile males exhibit an extremely narrow range in their DNA values while the 21 infertile males show a striking variation with most of the DNA values

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Fig. 1. Range of amounts of DNA (microspectrophotometry) in cytologically normal spermatozoa of 21 human males with proven fertility and 21 human males with suspected infertility. N⁰ 850.

Case No.	Mean amount of DNA per sperm	
	Proven fertility group	Suspected infertility group
1	1.18±.01	.32±.02*
2	1.18±.02	.73±.02
3	1.19±.01	.78±.05
4	1.19±.02	.80±.02
5	1.20±.02	.84±.02
6	1.20±.02	.86±.02
7	1.20±.02	.88±.02
8	1.21±.02	.89±.01
9	1.21±.03	.89±.02
10	1.22±.02	.90±.02
11	1.22±.02	.90±.02
12	1.23±.01	.92±.02
13	1.23±.02	.93±.03
14	1.23±.01	.94±.02
15	1.24±.01	.97±.02
16	1.24±.02	.97±.03
17	1.24±.03	1.00±.01
18	1.25±.02	1.05±.03
19	1.25±.02	1.18±.02
20	1.29±.01	1.39±.02
21	1.30±.01	1.58±.03*

N⁰ = Number of spermatozoa measured. * Rarely encountered.

significantly lower than those of the fertile group. That the constancy of the DNA content in the spermatozoa of fertile men can really be used as a baseline for comparison is brought out by the examination of the data in fig. 2. Here one sees the distribution curves of the DNA values in 563 *individual* spermatozoa of 21 fertile males, in 968 *individual* spermatozoa of 13 fertile males and in 630 *individual* spermatozoa of 8 fertile males obtained in studies done at different times over a three year period. There is not only complete agreement between the mean DNA values in the 3 samples but the 3 distribution curves of the individual DNA values in all the fertile groups are nearly identical. The contrasting low DNA values found in the spermatozoa of the infertile males as compared with the normal DNA values are especially remarkable since in both groups only spermatozoa of cytologically normal appearance were selected for the DNA measurements. In other words no abnormal forms occurring in each sample were used for DNA determinations and consequently the measured spermatozoa of the fertile and infertile males

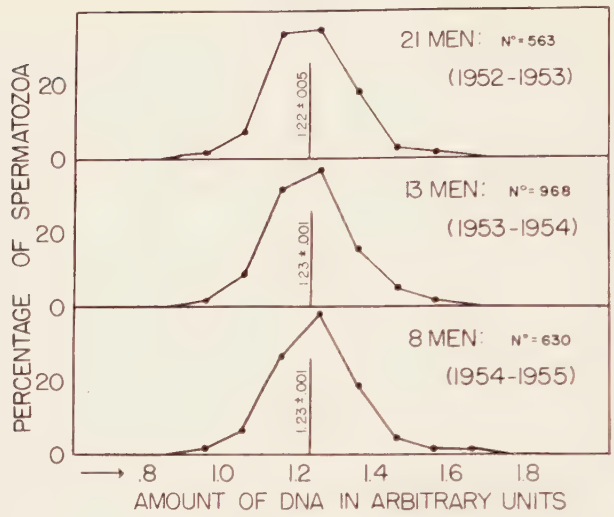

Fig. 2. Amount of DNA (microspectrophotometry of *Feulgen* reaction) in individual spermatozoa of fertile men. Studies done at different periods (1952–1955). N° = number of spermatozoa measured. Mean amounts of DNA represented by vertical line.

could not be distinguished cytologically. This statement is also confirmed by the data presented in fig. 3 in which the sizes of the measured spermatozoa of both groups are compared. Here one can see that the mean nuclear diameters for spermatozoa of fertile men is 2.53 micra which is exactly the same size as that of the infertile group, a result which is also expressed in the two identical distribution curves of the individual nuclear diameters.

In view of this constancy of DNA in fertile men and the low DNA which was found only in spermatozoa of infertile men, it seemed pertinent to investigate the following question: How characteristic is the DNA value in the spermatozoa for an individual over a period of time? In other words, do the spermatozoa of seminal fluids taken at different intervals always show the same normal DNA values for the fertile males and

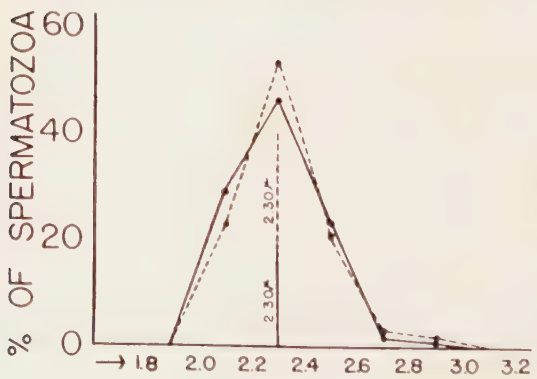

Fig. 3. Nuclear diameters in arbitrary units. Nuclear diameters of 912 spermatozoa of fertile men, and 1772 spermatozoa of men with suspected infertility.
—— Spermatozoa of fertile men.
- - - - Spermatozoa of men with suspected infertility.

always a low DNA value for the same infertile male? In figs. 4 and 5 a characteristic example for each, a fertile and an infertile male, are presented. It can be seen that 12 repeat samples within a period of two years did not show any fluctuations in the DNA values of the spermatozoa from a fertile male: all the spermatozoa contained the normal haploid DNA value. This stability within a fertile individual is strikingly different from the DNA value of a repeat sample in an infertile individual (fig. 5). Here fluctuations in the DNA values from as low as 0.3 up to the normal DNA values occurred over a similar period of time. In a study of 35 infertile males in which repeat seminal fluids were examined for their DNA

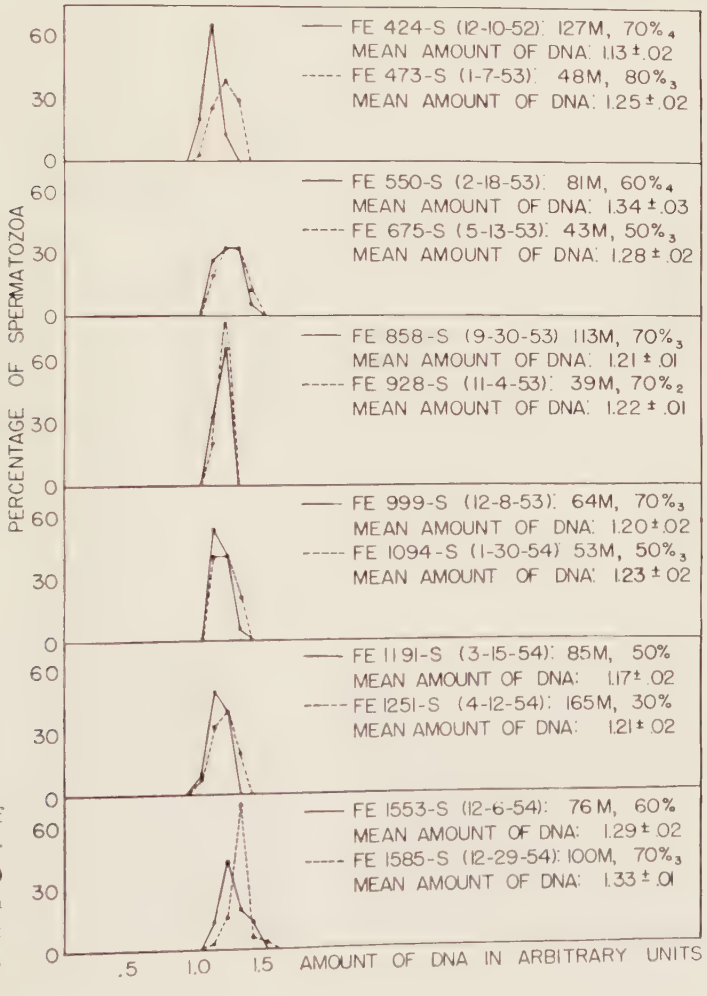

Fig. 4. Amount of DNA (microspectrophotometry) in the spermatozoa of the same male at different intervals.

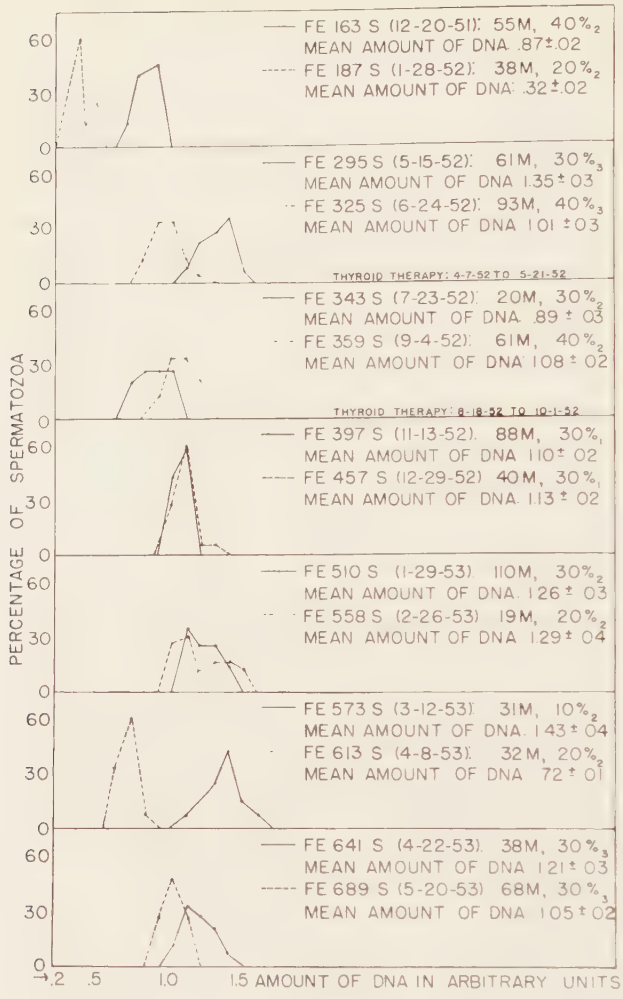

Fig. 5. Amount of DNA (microspectrophotometry) in the spermatozoa of the same male at different intervals.

content essentially the same picture was obtained, that is a low DNA value in an infertile male is by no means a permanent feature but may vary to a marked degree, while 25 fertile males in repeat specimens which were studied showed without any exception always the normal haploid DNA content (*Leuchtenberger, Weir, Schrader* and *Murmanis* [1955]). Of interest is, however, that in infertile males the majority of the repeat samples gave abnormal DNA values; for example, from 148 specimens 85% showed too low DNA while only 15% showed a normal DNA value. On the other hand all 114 specimens of the fertile group gave the normal DNA value.

In view of the low DNA values found only in the spermatozoa of the infertile group the question arose whether this low DNA value is due to a loss of DNA either from the mature sperm per se or from the germ cells, or whether there is perhaps a faulty DNA synthesis during the spermatogenesis of the infertile males. In an attempt to answer these questions, DNA determinations were made in the spermatogenic cells of testicular biopsies from fertile and infertile men. Figure 6 shows an example of typical DNA data obtained for the spermatogenic cells from fertile and infertile men. For the fertile men, spermatids, secondary and primary spermatocytes show DNA values and ratios in accordance with the chromosomal numbers, namely haploid, diploid and tetraploid respectively. The infertile men who had low DNA values in spermatozoa of repeated seminal fluids showed significantly lower DNA values in all the spermatogenic nuclei, but the ratios of 1:2:4 between spermatids, secondary and primary spermatocytes were always maintained in spite of the deficient DNA values present as early as in the primary spermatocytes (*Leuchtenberger, Leuchtenberger, Schrader* and *Weir* [1956]). On the basis of these data in the spermatogenic cells it seems justified to state that the low DNA values found in the spermatozoa of infertile males are not due to a loss from the mature sperms but that the deficiency can be traced back to a stage as early as the primary spermatocytes.

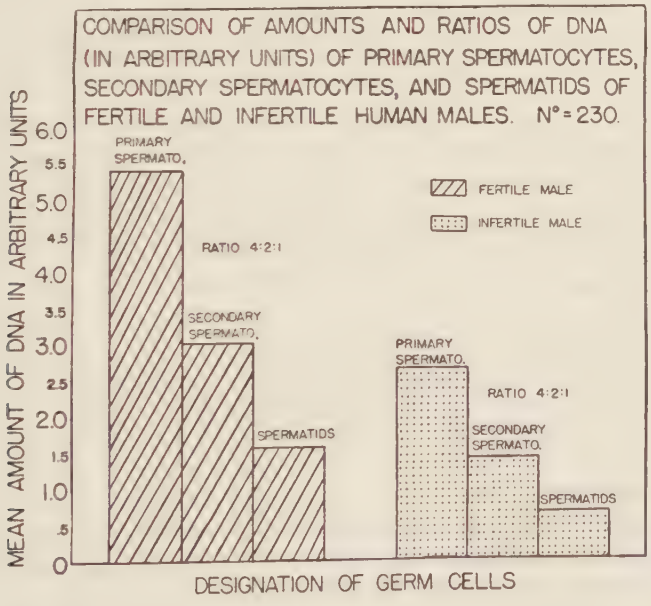

Fig. 6

The faulty amounts of DNA in the spermatogenic cells and mature spermatozoa in the infertile group as contrasted with the strikingly constant normal amount of DNA in these cells in fertile men justifies the conclusion that at least one type of male infertility may be caused by a DNA deficiency. The difference between the two groups seems all the more significant since clinical, histological and cytological examinations did not reveal any essential differences between them with the exception, of course, of the existing sterility. Furthermore, this DNA deficiency prevails, no matter how the usual criteria for the examination of the seminal fluids appear. In our studies, the faulty amount of DNA is found even in those cases where the count is high, the motility good and the morphology normal. Similarly, in the cases we have classified as normal from the standpoint of the DNA content, the counts may be low and the morphology somewhat abnormal.

The correlations between DNA constancy and fertility and variable DNA deficiency and infertility is actually not so surprising if one takes into account the fact that DNA is an essential constituent of the chromosomes and the genetic material. Therefore the use of DNA analysis would seem indicated as an added diagnostic tool for the study of human fertility problems.

REFERENCES

Leuchtenberger, C.: Critical Evaluation of Feulgen Microspectrophotometry for Estimating Amount of DNA in Cell Nuclei, Science *120*, 1022, 1954.

Leuchtenberger, C., R. Leuchtenberger, F. Schrader and *D. R. Weir:* Reduced Amounts of Desoxyribose Nucleic Acid (DNA) in Testicular Germ Cells of Infertile Men with Active Spermatogenesis. Lab. Invest. *5*, 422, 1956.

Leuchtenberger, C., F. Schrader, D. R. Weir and *D. P. Gentile:* The Desoxyribosenucleic Acid (DNA) Content in Spermatozoa of Fertile and Infertile Human Males. Chromosoma *6*, 61, 1953.

Leuchtenberger, C., D. R. Weir, F. Schrader and *L. Murmanis:* The Desoxyribose Nucleic Acid (DNA) Content in Spermatozoa of Repeated Seminal Fluids from Fertile and Infertile Men. J. Lab. clin. Med. *45*, 851, 1955.

Discussion:

P. C. Koller (London): The ratio of DNA 4:2:1 for the primary and secondary spermatocytes being the same in normal fertile and infertile men, seems to suggest that the low DNA content may be due to other causes than a loss in the chromosome complement.

T. C. Carter (Harwell): Dr. *Leuchtenberger* expressed her DNA measurements in arbitrary units. Has she any measurements in absolute units?

C. Leuchtenberger (Cleveland): Yes, a factor of 2×10^{-9} mgm. will convert these arbitrary units to absolute amounts of DNA.

Doepfmer, R.: Acta genet. *6*, 279–282, 1956

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UNTERSUCHUNGEN ÜBER DIE MORPHOLOGIE UND DIE MOTILITÄT VON HODENSPERMATOZOEN

Von R. DOEPFMER

Die eigenen Untersuchungen galten der Fragestellung, ob Hodenspermatozoen in einer geeigneten Extraktionsflüssigkeit ebenso wie Ejakulationsspermatozoen funktionstüchtig sein können und inwieweit sie im Hinblick auf ihre Morphologie und Motilität den Ejakulatspermatozoen ähnlich sind. Dabei sollte durch experimentelle Versuche vor allem geklärt werden, ob die Annahme von *Iwanoff* auch theoretisch gerechtfertigt ist, daß Hodenspermatozoen bereits ihre volle Bewegungs- und Befruchtungspotenz besitzen, was angeblich durch Inseminationen mit Hodenspermatozoen nach *Götze* und *Iwanoff* in Tierversuchen und nach *Adler* und *Makris* auch beim Menschen bewiesen sein soll. Diese Frage ist deshalb von so großer Bedeutung, weil durch neue Erkenntnisse über Hodenspermatozoen möglicherweise den Patienten geholfen werden kann, die durch einen kompletten Verschluß der samenabführenden Wege wegen einer postinfektiösen Induration des ganzen Nebenhodens oder wegen Mißbildungen nicht operativ rekanalisiert werden können.

Nach den bisherigen Erkenntnissen sollen die Spermatozoen ihre volle Funktionstüchtigkeit erst nach der Nebenhodenpassage erlangen, wobei nach *Redenz* der Reifungsvorgang durch die von den Nebenhodensekreten induzierte Wirkung und nach *Young* nur durch einen innerhalb des Spermatozoons induzierten Reifungsvorgang und somit also nur durch eine Weiterentwicklung auf dem 7 m langen Wege durch die Gänge des Nebenhodens erfolgen soll.

Die experimentellen Untersuchungen wurden an Ratten-, Meerschweinchen-, Hunde- und Bullen-Hodenspermatozoen sowie an menschlichen Hodenspermatozoen durchgeführt. Durch ein besonderes Extrak-

tionsverfahren konnten aus 1 g menschlichen Hodenparenchyms bis zu 3 Millionen Spermatozoen pro cm^3 ausgeschwemmt werden.

Bei den morphologischen Untersuchungen fanden sich unter den ausgeschwemmten Hodenspermatozoen bei 40–50% vergrößerte Köpfe und bei 20–30% abartige Formen und bei etwa 50% sogenannte Protoplasmatropfen. Mit den bekannten Färbeverfahren konnten im Lichtmikroskop bei 30% der Hodenspermatozoen keine Unterschiede gegenüber Ejakulatspermien festgestellt werden. Die elektronenmikroskopischen Untersuchungen gestalteten sich wegen der kleinen Zahl der Spermatozoen und wegen der die Spermatozoen überlagernden Zellen der Samenreifungsreihe, Gewebszellen und Zellen des Blutes sehr schwierig. Doch konnte im Aufsichtsbild elektronenoptisch ebenso wie im Lichtmikroskop kein Unterschied gegenüber Ejakulatspermatozoen nachgewiesen werden. Auffällig waren die häufigen, besonders im Bereich des Zwischenstückes aber auch am Kopf nachweisbaren, zytoplasmatischen Ausstülpungen und Einrisse der äußeren Membran. Die nachgewiesenen zytoplasmatischen Ausstülpungen konnten nur in einem Teil der Fälle als sogenannte Protoplasmatropfen angesehen werden. Diese häufigen Veränderungen können nun bedingt sein

1. durch artifiziell verursachte Schäden bei der Präparation,
2. durch fermentative Zersetzungsvorgänge, da oft die Hoden erst einige Stunden post mortem untersucht werden konnten, und
3. durch eine leichtere Verletzbarkeit der noch nicht voll widerstandsfähigen Membran.

Mit der von *Schultz-Larsen* erstmals angegebenen Methode der Darstellung geschnittener Spermatozoen im Elektronenmikroskop konnten die von *Schultz-Larsen*, *Hammen* und *Carlsen* aufgezeigten Befunde an Ejakulatspermatozoen (Galea capitis, Vakuolen und Mitochondrien) auch an Hodenspermatozoen bestätigt werden.

Bei der Prüfung der Motilität unterschieden wir bei den in den zu prüfenden Extraktionsflüssigkeiten suspendierten Hodenspermatozoen zwischen der Quantität und der Qualität der Bewegung. Unter der Quantität der Bewegung verstehen wir das Verhältnis der Zahl der beweglichen zu den unbeweglichen Spermatozoen. Als Qualität bezeichnen wir den Grad der Bewegungsintensität der Spermatozoen, die sich am lebhaftesten bewegen.

Auf Grund systematischer Untersuchungen der Ionenwirkung auf die Bewegung von Hodenspermatozoen ergab sich nach Prüfung von etwa 200 Lösungen der überraschende Befund, daß die physiologische Ammoniumchloridlösung die Wirkung aller geprüften Lösungen im Hin-

blick auf die Quantität, und besonders die Lebensdauer und die Qualität beträchtlich übertraf. Die mit der physiologischen Ammoniumchloridlösung erreichte Qualität der Bewegung zeigte eine deutliche Progression und eine besondere Art der Motilität bei einer kleinen Zahl, jedoch in keinem Falle den gleichen Intensitätsgrad der Qualität wie bei Nebenhoden- und Ejakulatspermatozoen.

Besonders erwähnenswert ist die Tatsache, daß sich in physiologischer Ammoniumchlorid-Lösung bei Zimmertemperatur und einem pH-Wert von 6,2–6,8 erstmals eine Dauer der Bewegung von 56 Stunden nachweisen ließ, während die längste bisher in der Literatur veröffentlichte Dauer der Bewegung von Hodenspermatozoen nach *Belonoschkin* 3 Stunden betrug.

Der pH-Wert, die Temperatur, die Zusammensetzung und Konzentration der Elektrolyte waren im Hinblick auf die Auslösung, Quantität, Qualität und Dauer der Spermatozoenbewegung mittelbar und unmittelbar weitgehend voneinander abhängig und beeinflußten sich gegenseitig.

Auf Grund der allgemeinen Vorstellung über die physikalisch-chemischen Zustände und Abläufe in den Zellen sind mehrere Angriffspunkte für anorganische Ionen und insbesondere für das NH_4-Ion denkbar, die in der Beeinflussung von geformten und ungeformten Zellbestandteilen sowie in energieliefernden und vorhandene Energieformen umwandelnden Mechanismen bestehen. Die Wirkung der Ammonium-Ionen auf die – möglicherweise – in ihrer Stoffwechselleistung noch nicht vollentwickelten Hodenspermatozoen läßt sich so deuten, daß diese Ionen in dem offenbar noch ungebahnten Fermentmechanismus einen eingleisigen, energieliefernden Stoffwechselvorgang erzwingen und so die erhöhte, im Hoden ohne die Ammoniumchlorid-Wirkung noch nicht vorhandene Bewegungssteigerung ermöglichen.

Die Stoffwechseluntersuchungen lassen sich an Hodenspermatozoen nicht in der gleichen Weise wie an Nebenhoden- und Ejakulatspermatozoen durchführen, da sich Hodenspermatozoen nicht von den Zellen der Samenreifungsreihe und den übrigen Hodengewebsbestandteilen in den Suspensionsflüssigkeiten trennen lassen.

Zusammenfassend läßt sich sagen, daß sich auf Grund unserer Untersuchungen folgende Unterschiede zwischen Hodenspermatozoen und Ejakulatspermatozoen der verschiedenen Tiere und des Menschen aufzeigen ließen:

1. Eine erhöhte Verletzbarkeit der Zellmembran besonders im Bereich des Mittelstücks, die sich elektronenoptisch durch Protoplasma-Austritt nachweisen ließ.

2. Das Vorhandensein sogenannter Protoplasmatropfen bei etwa 50% der untersuchten Hodenspermatozoen.

3. Die Zahl der normalgeformten Hodenspermatozoen betrug nur etwa 30% und die Zahl der Spermatozoen mit vergrößerten Köpfen 40–50%.

4. Die Motilitätssteigerung durch bestimmte Ioneneinflüsse war nur bei einer kleinen Zahl erzielbar.

5. Die starke Motilitätssteigerung durch die physiologische Ammoniumchloridwirkung weist ein von der normalen Motilität abweichendes Bild auf und ist offenbar nicht als eine toxische Stimulation aufzufassen, da die Dauer der Motilität bis zu 56 Stunden betrug.

Inwieweit sich diese Ergebnisse als Möglichkeit zur Nachahmung des Nebenhodeneffekts auswerten lassen, wird erst durch systematische Inseminationsversuche mit Hodenspermatozoen beantwortet werden können, die in den gefundenen Extraktionslösungen suspendiert sind.

Der praktische Wert der Motilitätsstudien liegt in der Erkenntnis, daß jeder operative Versuch der Anlegung einer Hoden-Ductus deferens-Anastomose – unabhängig von den technischen Schwierigkeiten – von falschen Voraussetzungen ausgeht, da Hodenspermatozoen bei Umgehung des Nebenhodens ohne die Schaffung eines optimalen Milieus in vivo in kurzer Zeit zugrunde gehen.

Die elektronenmikroskopischen Untersuchungen wurden in dem Universitäts-Hygiene Institut Würzburg (Direktor: Prof. Dr. *C. Sonnenschein*) von Frau Dr. med. *E. Mölbert* durchgeführt.

Schultz-Larsen, J. and *R. Hammen*: Acta genet. *6*, 282, 1956

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THE SUBMICROSCOPIC MORPHOLOGY OF HUMAN SPERMATOZOA

By J. SCHULTZ-LARSEN and R. HAMMEN

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THE ROLE AND IMPORTANCE OF MUTATION, VARIATION AND ADAPTATION IN MALIGNANT GROWTH[1]

By P. C. KOLLER

Introduction

It is common experience that in patients tumours of the same types and which are growing at similar sites, respond differently, when exposed to ionising radiation or to chemotherapeutic agents. In order to throw some light on the cause of the variable response, the dynamics of malignant growth have been studied by the author in a large number of human and experimental cancers. As a result of these studies it has been disclosed that the growth characteristics and the degree of malignancy of tumours are determined primarily by three processes; mutation, selection and adaptation. These phenomena have been the subject of numerous genetical investigations during the last decade and much information relevant to cancer has been obtained by the studies of populations of microorganisms. It is the author's aim to discuss some phenomena of tumour behaviour in the light of modern genetical concepts in order to show that a much better understanding can be gained of the problems and difficulties which are involved in the control and therapy of cancer.

[1] This investigation has been supported by grants to the Royal Cancer Hospital and Chester Beatty Research Institute from the British Empire Cancer Campaign, the Jane Coffin Childs Memorial Fund for Medical Research, the Anna Fuller Fund and the National Cancer Institute of the National Institutes of Health, U.S. Public Health Service.

Heterogeneity of Tumours

The cell population of tumours is brought about by the multiplication of the malignant cells. It might be expected therefore, that the cell components of malignant tissue are identical owing to the fact that they were reproduced by mitosis, which normally yields only genetically identical daughter cells. The author found however, contrary to expectation that the cell population of tumours contains numerous cell variants. They can be identified by their morphological features which very likely are only the visible manifestations of much more complex metabolic differences. It was furthermore found that the cell population of malignant tissue is in a dynamic equilibria. Under environmental influences the component cells respond differently and compete amongst themselves for survival. The outcome of this process is the elimination and selection of cell variants which not only changes the histological structure, but very often alters the degree of malignancy of the original tumour.

In the selection of cell variants, the reaction by the host plays an important role. It has been observed that in spite of the similarity of antigenic constitution between primary tumours and host tissue in which they arise, local inflammatory reaction usually accompanies the invasion of a growing tumour. The reaction in the tumour bed may be only a secondary event, brought about by the toxic breakdown products which are always present in the poorly vascularised tumour tissue. On the other hand evidence has been obtained to suggest that the inflammatory reaction is one of the defence mechanisms of the host against the invading tumour and it has a definite genetic basis. Instances are known in which hypersensitivity of certain tissues is genetically determined. Thus a close association has been found between blood group A and the susceptibility to inflammatory response in the gastric mucosa favouring the development of gastric carcinoma in man. The author's study of a few family histories suggests that the intensity and time of onset of inflammatory reaction is genetically controlled.

Transformation of Tumours

Sarcomatous transformation of carcinoma has been often reported; it is the best example of alteration in the tumour cell population. The original tumour has been composed of both epithelial cells and fibroblasts and the transformation is the result of an overgrowth of the latter under selection pressure by the host. Regional differences in the histological structure of tumours and the changing of well-differentiated carcinoma

into an anaplastic type have been observed by the author in several human tumours. The analysis of these events led to the conclusion that tissue reaction exerted by the host is an extremely powerful agent which can change the cell composition of tumours. Furthermore it was confirmed that the change is not only morphological, but it is very often associated with a greater degree of malignancy.

The role which the environment plays in selecting cell variants has been investigated in a transplantable rat tumour induced by an aromatic nitrogen mustard (*Koller* [1953]). This particular tumour has shown chromosome abnormalities in a very high proportion of the dividing cells, the most conspicuous abnormality being the chromosome bridges during anaphase. In the primary tumour the incidence of cells with chromosome bridges was 68 per cent, in the transplant generation it was reduced and stabilised around 20 per cent. When, however, the tumour was cultured *in vitro* or grown as ascites, cells with chromosome bridges were absent. It seems that the environmental conditions in tissue culture and ascites did not favour the multiplication of cells with dicentric chromosomes and as a consequence the abnormal cells have been replaced by normal ones. It is very likely that the hypotetraploid HeLa strain of human carcinoma which is maintained in tissue culture, has been transformed from the diploid state by environmental conditions.

Adaptation and Mutation

Change can affect physiological behaviour as a result of which, the tumour will show increased virulence, growth rate and altered specificity in transplantation. The change may be brought by cell adaptation or mutation. Thus the increased transplantability of a particular mammary carcinoma in mice which was brought about by passing the tumour through F_1 hybrid between susceptible and resistent strains, was interpreted by *Barrett* and *Deringer* [1950] as an example of physiological adaptation. On the other hand, the change in transplantability can be attributed to alteration in the constitution of the histocompatibility genes due either to gene mutation at the H-locus or to change in the number or structure of chromosomes. Both events are known to occur in tumours. Mutation in histocompatibility genes has been reported by several investigators and the influence of numerical changes in chromosome constitution upon the degree of antigenecity of tumours has been demonstrated (*Snell* [1953], *Hauschka* and *Levan* [1953]). The numerical change is always towards polyploidy, and represents a drastic rearrangement of the genic equilibrium which would ease the selection pressure exerted

by the host in every homotransplantation. The intensive study of karyotypes of diverse tumours—which is in progress now—can be expected to throw further light on the adaptive value of cell-heterogeneity in tumour transplantation.

Hormone Dependent and Autonomous Tumours

The process by which mutation and selection operate in the development of malignant growth can be profitably studied in tumours whose origin and transplantability is controlled by endocrine glands. Thus for instance the extremely rare tumour of the thyroid, can be induced in mice and rats by depleting the circulation thyroid hormone and can be carried by transplantation in conditioned animals in which the same hormone is depressed. It is however a frequent occurrence that such hormone dependent tumours after a number of serial transfers become autonomous. The transformation is due to selection of particular cell variants, brought about by the reaction of the host which operates in homo-transplantation.

Selection in Ascites Tumours

The cell populations of ascites tumours have been found to be especially favourable material to study the dynamics of malignant growth. During the last few years the bahaviour of chromosomes in these tumours has been intensively studied and the investigations provided further evidence that the cell population of malignant growth is heterogenous. As an example we may mention the experiment of *Kaziwara* [1954] in which a polyploid subline of *Ehrlich* ascites has been established by the intraperitoneal injection of low cell dosage taken from the hyper-diploid strain. It seems that small cell dosage permits the host to build up at least temporary effective defences, which select the polyploid cells with the least immune response.

The analysis of effusions in human carcinomatosis produced more evidence to show that the behaviour of the tumour cells is greatly influenced by the environmental conditions in the ascitic fluid (fig. 1). It has been possible to detect the mechanism by which changes have been brought about in the chromosome constitution of the malignant cells. The depletion of nutrients and the accumulation of degradation products of cellular decay in the ascitic fluid produce mitotic abnormalities, particularly spindle irregularities which results in cells differing in chromosome number and structure. The cell variants are exposed to selection during which process the anaplastic type is greatly favoured. It has been

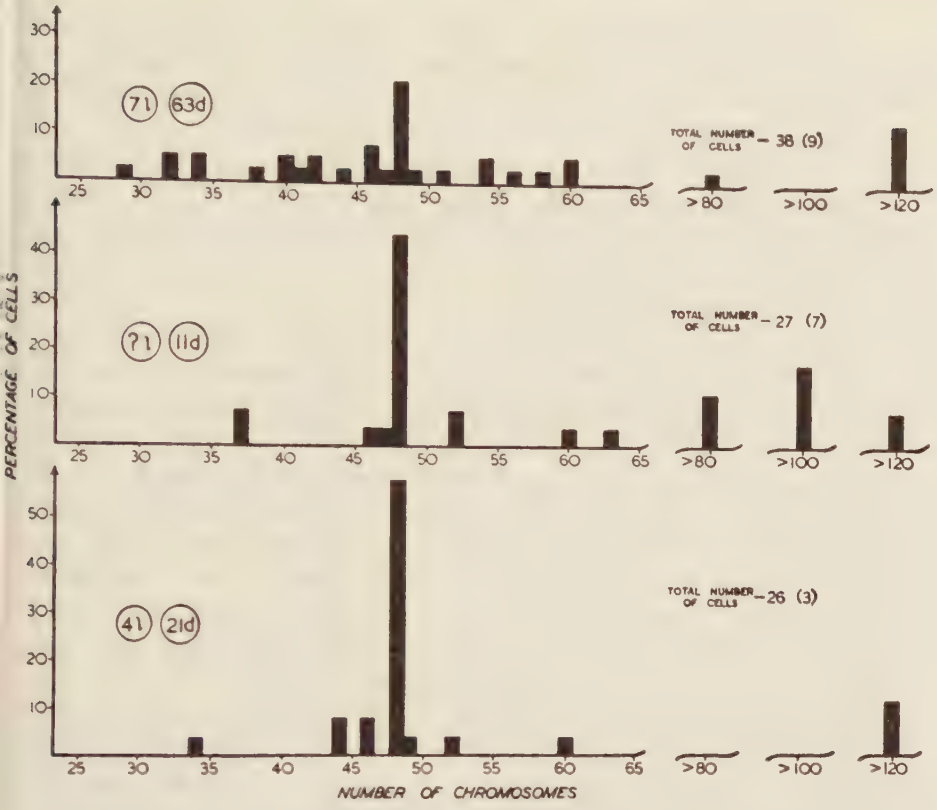

Fig. 1. Histograms showing the frequency of cells with different chromosome numbers in three successive peritoneal effusions of a patient with carcinoma of the cervix. Numbers in circles show the quantity of fluid (in litres) and the time interval in days between the successive paracenteses. Numbers in brackets indicate the number of cells in which the counting of chromosomes was uncertain.

found that the ascites is a condition in which the differentiated tumours undergo rigorous trial for fitness, adaptation and survival and that the evolutionary events lead towards the establishment of the anaplastic cell type as the predominant of the new tumour.

Development of Resistance to Treatment

The few examples described above, were chosen to demonstrate the important facts that (i) the cell population of tumour tissue is heterogeneous and that (ii) particular cell variants are selected by environmental conditions.

A very similar behaviour has been observed in both human and experimental tumours, which have been exposed to ionising radiation or chemotherapeutic drugs. During the studies of radiation response in human epidermoid carcinoma, one tumour was found in which a high number of polyploid cells survived the radiation injuries and became a source of a new tumour. The recurrent carcinoma contained a large number of polyploid cells, and proved to be resistent to radiation. Several other instances are known in which transplantable mouse tumours became resistant to X-rays by selection.

The present author found that in some cases the malignant cell population of human effusions, which was treated with radioactive colloid gold (Au 198), had been almost completely destroyed while in others the proportion of tumour cells had been greatly reduced with a change in cell polymorphism. The most significant change after treatment with Au 198 was the great increase in the proportion of diploid cells. If these cells represent the "stem cells" of the ascites i.e. the cells that supply the main source of the population of the new effusion, then observation seems to indicate that the "stem cells" have been selected because they are most resistant to radioactive colloid gold than cells with either less or more chromosomes than the diploid number (fig. 2).

A similar type of reaction has been found in both human and experimental tumours which have been treated with cytostatic drugs e.g. nitrogen mustard, amethopterin, myleran etc. *Law* [1954] reported that cells of mouse leukaemia after exposure to a folic acid antagonist, became resistant to the drug. He was able to demonstrate that the resistant cells were not due to mutation induced by the drug, but that they were already present in the tumour tissue and the drug acted only as an agent of selection. It is common experience that in the chemotherapy of human leukaemias with HN2, or myleran, the cell populations of the marrow become resistant to these drugs, with fatal consequences to the patient. Oestrogens can also act in a similar manner. The present author observed the transformation of a well-differentiated carcinoma of the prostate which was treated with stilboestrol. After 18 months of treatment, the carcinoma became an anaplastic tumour which failed to respond to further treatment. In these instances just mentioned, we are dealing with the phenomenon of drug-resistance, which is similar to that often encountered in genetical experiments with micro-organisms.

Because the composition of cell population in tumours undergoes changes due to selection pressure, it can be expected that the same tumour might respond differently to the same drug when it is tested at different

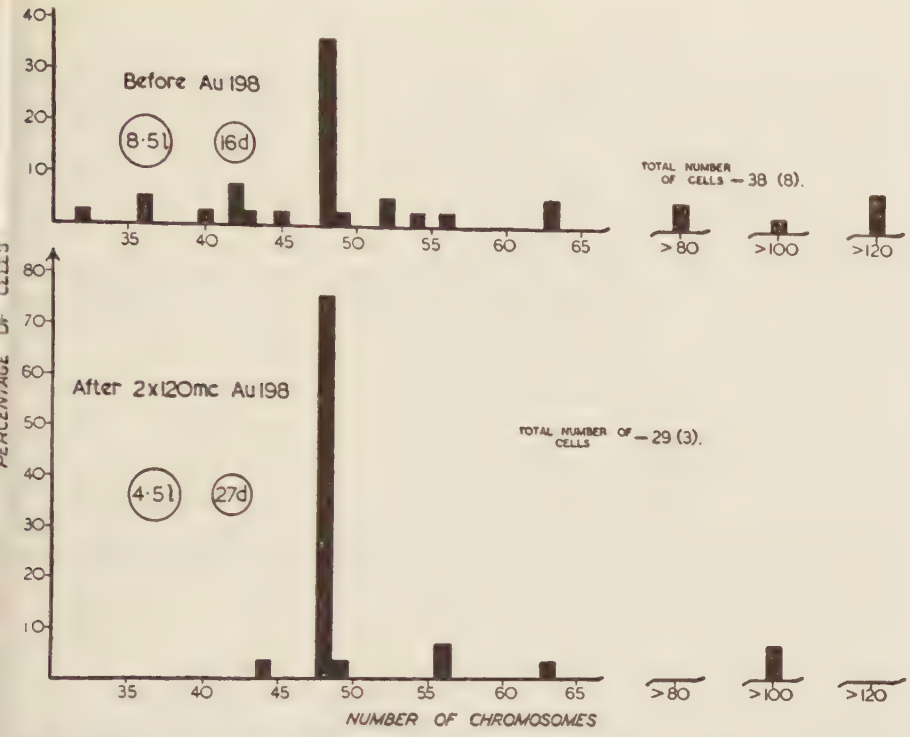

Fig. 2. Cell polymorphism in peritoneal effusions before and after treatment with Au 198.

times or against tumours which were carried in strains different from those originally used as a host. Resistance of a previously susceptible tumour to a drug has been encountered in several instances which could be attributed to change in cell composition of the tumour. The change might have been brought by selection or mutation; in most instances however, it is not possible to determine which of the two processes is responsible for changing the susceptible tumour to one which is resistant to the drug. Many of the agents used in the therapy of cancer, are known to be mutagenic (e.g. HN2, TEM. urethan, myleran etc.), therefore the possibility exists that resistance to the drug, at least in some cases, is due to mutation. In this connection, it should be also emphasised that these substances have been found to induce germinal mutations and structural changes in the chromosomes with genetical effects (e.g. inversion, translocation). Although the danger of increasing the load of deleterious mutations in man is small, yet it is present when the mutagenic drugs are employed in the therapy of cancer. For that reason their use should be weighed against the long term genetical consequences which are involved.

Conclusion

The few examples discussed above are aimed to illustrate the fact that within tumours, mutation, selection and adaptation are continuously in action. The evolutionary drive is towards diversity and the cell variants result, represent potentialities for the development of tumours with greater malignancy. If further advance is aimed at in the control of malignant disease, the importance of the biological factors and the role which they play in determining the growth characteristics of tumours, must be taken into consideration by all those who are engaged in the therapy of cancer.

REFERENCES

1. *Barrett, M. K.* and *M. K. Deringer:* An induced adaptation in a transplantable tumor in mice. J. nat. Cancer Inst. *11*, 51–59, 1950.
2. *Hauschka, T. S.* and *A. Levan:* Inverse relationship between chromosome ploidy and host specificity of sixteen transplantable tumors. Exp. Cell Res. *4*, 457–467, 1953.
3. *Kaziwara, K.:* Derivation of stable polyploid sublines from a hyperdiploid *Ehrlich* ascites carcinoma. Cancer Res. *14*, 795–801, 1954.
4. *Koller, P. C.:* Dicentric chromosomes in a rat tumour induced by an aromatic nitrogen mustard. Heredity *6*, Suppl., 181–196, 1953.
5. *Koller, P. C.:* Cytological variability in human carcinomatosis. Ann. N. Y. Acad. Sci. *63*, 793–816, 1956.
6. *Law, L. W.:* Genetic studies in experimental cancer. Advanc. Cancer Res. *2*, 281–352, 1954.
7. *Lüers, H.:* Zur Frage der Erbschädigung durch tumortherapeutische Cystostatica. Z. Krebsforsch. *60*, 528–535, 1955.
8. *Snell, G. D.:* Transplantable tumors, in *F. Homburger* and *W. H. Fishman:* Physiopathology of Cancer, p. 338–391. Cassel & Co., London 1953.

Discussion:

C. E. Ford (Harwell): Although my experience cannot be compared with Professor *Koller's* and is almost confined to primary radiation induced neoplasms of the reticuloendothelial system in mice. I have come to a very similar conclusion. In the cases my colleagues and I have studied most are composed of cell populations all, or nearly all, of the component cells of which differ from normal as regards their chromosomes. Each appears to be unique in respect of the following cytological properties taken collectively 1. Stem line, or modal, chromosome number; 2. variation in number about the mode; and 3. the presence of one or more characteristic abnormal chromosomes in the stem-line chromosome sets of some.

Leuchtenberger, Cecilie, R. Leuchtenberger and *Ethel Lieb*: Acta genet. *6*, 291–297, 1956

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STUDIES OF THE CYTOPLASMIC INCLUSIONS CONTAINING DESOXYRIBOSE NUCLEIC ACID (DNA) IN HUMAN RECTAL POLYPOID TUMORS INCLUDING THE FAMILIAL HEREDITARY TYPE[1]

CECILIE LEUCHTENBERGER, R. LEUCHTENBERGER and ETHEL LIEB

Polypoid tumors in the large bowel occur very frequently especially in the human adult. The most common form is the benign adenomatous polyp which arises from the *Lieberkühn* glands. Polyps may be single or multiple, sessile, pedunculated or villous. Although practically the entire intestinal tract may be involved, the most common site is the colon, particularly in the rectal portion. Rectal polyps are of particular importance since malignant degeneration of such polyps is relatively frequent and may occur simultaneously at distant points in the colon. Another reason why polyps should command special attention is that there is one type which is hereditary. This disease called "familial intestinal polyposis" is inherited as a *Mendel*ian dominant trait and is characterized by the presence of a large number of polypoid tumors in the colon or rectum some of which may also undergo malignant transformation. The hereditary tendency of the disease was recognized as early as 1882 by *Cripps* and through extensive studies of intestinal polyposis families by *Dukes* [1930, 1952],

[1] This investigation was supported (in part) by research grant C-1814 from the National Institutes of Health, U.S. Public Health Service and in part by a grant from the Elsa U. Pardee Foundation. The authors gratefully acknowledge the kind cooperation of Dr. *R. T. McCarty*, Milwaukee, Wisconsin for providing material on the familial polyposis studies.

Gardner [1951, 1956], *Lockhart-Mummery* [1925], *McCarty* [1953] and others, the pattern of dominant inheritance has been well established.

The present report ist not directly concerned with the problem of familial polyposis and its genetic aspects, but deals with a peculiar feature observed in all types of rectal polypoid tumors. Nevertheless, the findings to be discussed here may have some bearing on the genetic aspects of the polyposis problem since they concern the behavior of desoxyribose nucleic acid (DNA) in cells of polypoid tumors. The universal presence of DNA in all nuclei of all the cells of every living organism has been known since *Miescher*'s work at the end of the last century [1897] and its significance as an important constituent of chromosomal and genetic material has been recognized for a number of years.

In contrast to this ubiquity of DNA in all the nuclei, the occurrence of DNA in the cytoplasm is encountered rarely and in special circumstances only. In humans, cytoplasmic DNA has not been observed in cells of normal tissues but cytoplasmic inclusions containing DNA have been found in some virus diseases of the human, such as molluscum contagiosum and smallpox. Therefore, it was a great surprise to find cytoplasmic inclusions containing DNA in the cells of both benign and malignant rectal polypoid tumors. This observation which was first reported for 63 cases of polypoid tumors by *Leuchtenberger* in 1954 has been extended to over 600 cases in the meantime and has been repeatedly confirmed. Without any exceptions the cytoplasmic inclusions were found in all cases of rectal polypoid tumors examined so far including the polyps of the hereditary type and the metastases arising from malignant polypoid tumors. The inclusions were not found in a control material of several hundred specimens, comprising a variety of human tissues with and without pathological lesions including polyps from other regions and also benign and malignant tumors from other regions (*Leuchtenberger*, *Leuchtenberger*, and *Davis* [1954]).

When sections of normal rectum and rectal polyps are stained by the *Feulgen* reaction, which is specific for DNA and are examined under the microscope at magnifications of approximately 600 or higher, the following observations can be made (fig. 1): In the glandular epithelial cells of the normal rectum only the nuclei are *Feulgen* positive which is in

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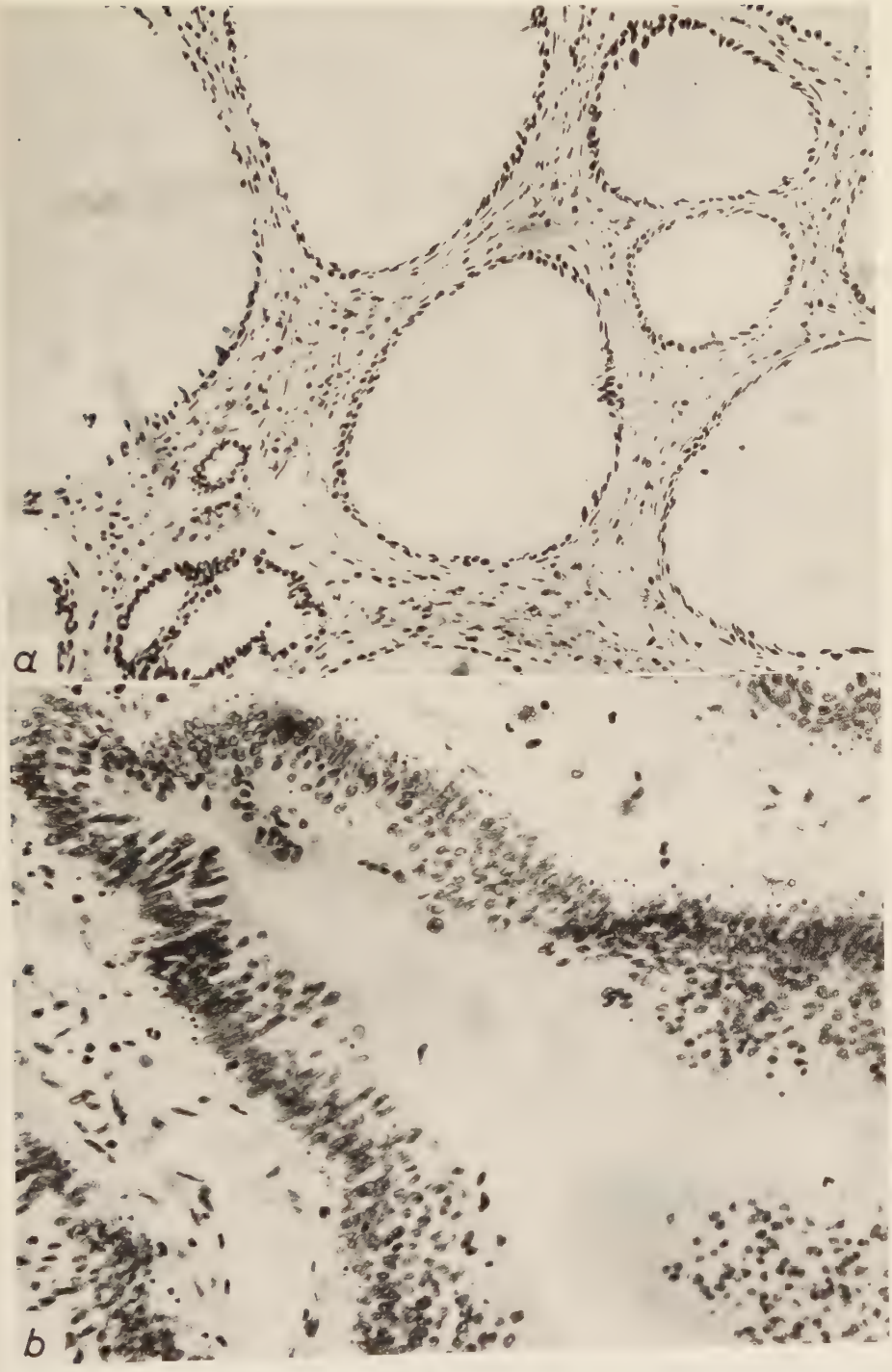
Fig. 1. (a) Glands from normal rectal mucosa showing cells with low cuboidal epithelium and regular disposition. (b) Glands from a rectal polyp showing proliferation and stratification of the epithelium. The cytoplasmic inclusions are clearly evident in the proximal portion of many of the cells. *Feulgen* reaction. Magnification, approximately 300 ×.


a
b

accordance with the fact that DNA is located only in the nuclei. The picture is considerably different if one examines the *Feulgen* stained slide of a rectal polyp. Here, in addition to the *Feulgen* positive nuclei, *Feulgen* positive material can be found in the cytoplasm. These cytoplasmic inclusions are mostly spherical, vary in size from less than 1 micron to approximately 2 micra, and occur in varying numbers either discretely or in conglomerations. Frequently they lie in pairs and sometimes are dumbbell shaped, suggesting fusion or possibly division. Very often the bodies are surrounded by a halo. The inclusions are usually located in the cytoplasm of the epithelial cells adjacent to the basal portion of the gland but are sometimes also found in the epithelial cells located toward the lumen. The cytoplasmic inclusions in the epithelial cells of the rectal polyps give without any exception, a *Feulgen* positive reaction. Since the bodies in nonhydrolyzed *Feulgen* controls are *Feulgen* negative and since the DNA in the bodies can be digested with a specific desoxyribonuclease, it can be concluded that the cytoplasmic inclusions in these polyps contain DNA. The cytoplasmic inclusions can also be easily stained with other basic dyes indicative of DNA and are consequently hematoxylin positive in a routine H and E examination. However, in the latter their identification is often obscured by precipitates or pigments.

In attempting to evaluate the nature and the possible significance of the cytoplasmic inclusions found in the glandular epithelium of the rectal polyps a number of possibilities were considered of which only the most pertinent can be discussed here.

The question of course arose at once whether the inclusions may not represent just nuclear debris derived from dying glandular epithelial cells or leucocytes. Against the possibility that the inclusions represent nuclear debris of dying epithelial cells is the fact that neither the cells containing the inclusions nor the adjacent cells show evidence of degenerative change. As a matter of fact, the absence of karyorrhexis, pycnosis and the presence of proliferation are characteristic features of the glandular epithelium in which the inclusions are most numerous. Mitotic figures are frequent and the nuclei are generally larger and give a more intense *Feulgen* reaction than those of non-tumorous glandular epithelial cells. Measurements of the DNA content by *Feulgen* microspectrophotometry (*Leuchtenberger* [1954], *Leuchtenberger, Doolin* and *Kutsakis* [1955] gave considerably higher DNA values in the nuclei of the epithelial cells of the rectal polyps than in the nuclei of normal rectum. That the inclusions may represent nuclear debris derived from invading leucocytes is also improbable. While some polyps show an inflammatory process with leucocytic infiltrations the great

majority of the polyps examined are essentially free of infiltrations, but the inclusion bodies are always present. In addition it is known to every experienced cytologist, that nuclear debris is characteristically polymorphic, of variable size and shape and dispersed more or less diffusely over the entire area. In contrast, the cytoplasmic inclusions in the rectal polyp are almost always spherical in shape, vary within a definite size range and are limited to the intracellular location. Finally, it hardly seems probable that degenerated nuclear material would be confined to one particular histological structure in one particular lesion.

The second possibility is that the inclusions may be material derived from chromosomes separated from the mitotic figure during abnormal mitosis. This also seems not too likely. In spite of careful cytological examination abnormal mitoses were very rarely encountered in the malignant and not found in the benign polypoid tumors. Furthermore, the inclusions were observed with the same frequency in the mitotic and non-mitotic areas. Preliminary electron microscopic examinations of the inclusions made at the Rockefeller Institute in collaboration with Dr. *Palade* showed an appearance entirely different from that of chromosomal material.

The third possibility, and the strongest one, is that these inclusions may be of viral origin. This concept is supported by several features. One feature consistent with the virus theory is that these bodies all contain DNA. It has been recently shown that nearly all viruses contain DNA. Furthermore, the cytological appearance and size of these bodies and their persistent *intracellular* location in one particular kind of lesion are all highly reminiscent of inclusions found in known instances of virus diseases. As a matter of fact, in studying the inclusions under the microscope at high magnification, one cannot help noting the striking similarity with the virus inclusions observed in an early stage of molluscum contagiosum infection. At this time, the inclusion bodies of molluscum contagiosum are still small *Feulgen*-positive spherical bodies in the cytoplasm of the skin epithelial cells and virtually look like the cytoplasmic inclusions found in the rectal polyps.

In order to investigate further the possible viral nature of these inclusions, transmission, tissue culture and electron microscope studies were attempted. In spite of a large series of experiments, efforts to propagate the human rectal polypoid tumors in the hamster cheek pouch according to the method of *Toolan* [1953] were unsuccessful. Sometimes, a prolonged survival of the original polyp transplant was observed and in such cases, the cytoplasmic inclusions in the cells were always present and at times, appeared even greater in number.

Since the tissue culture studies were started only recently, no data are available at the present time.

Although the electron microscope studies are still of a preliminary nature, the results obtained so far, are of sufficient interest to be mentioned here. If thin sections cut from osmic acid fixed and methacrylate embedded rectal polyps (according to *Palade* [1952]) are examined under the electron microscope, the inclusions in the cytoplasm show a double membrane and have a structure which is different from that of other cellular components such as nuclei, chromosomes, mitochondria or secretory granules. In favorable preparations, a honeycomb structure

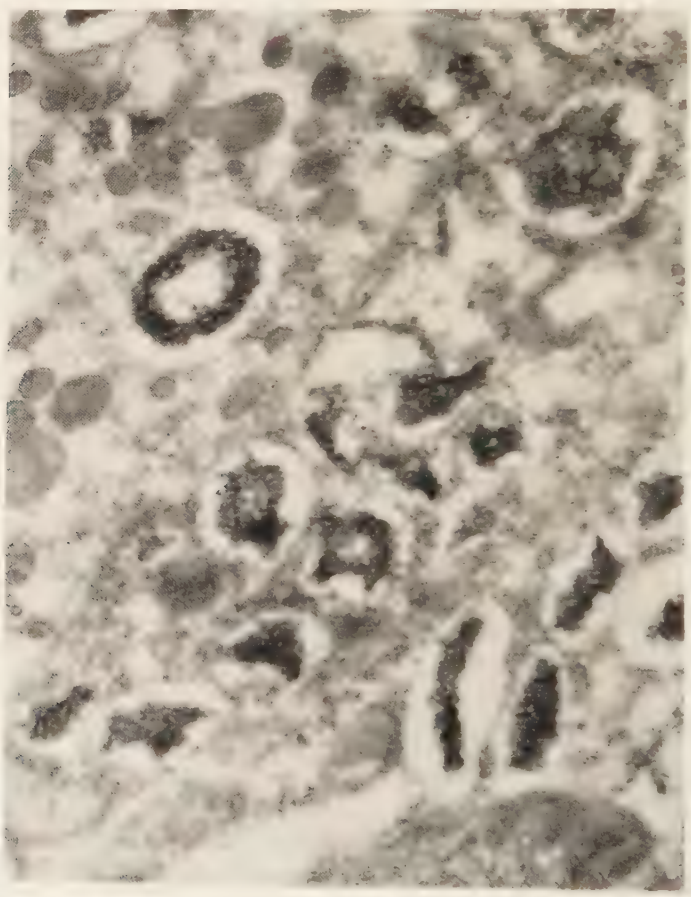


Fig. 2. Electron photomicrograph showing structure of inclusions found in the cytoplasm of a rectal polypoid tumor cell. Magnification, approximately 44,000 ×.

somewhat resembling the hexagonal packing of other viruses can be recognized within the inclusions.

It is of course realized that the studies done so far by no means establish the viral nature of these inclusions and that much more work is needed. On the other hand, it is hoped that these preliminary findings may stimulate further investigation into the nature of these inclusions. Regardless what the inclusions may turn out to be, they are a persistent feature of rectal polypoid tumors including the hereditary type and, as *Gardner* points out, "This relationship could very well represent the beginning for significant progress in the etiology of polyposis. In fact, it could lead to the discovery of a relationship between the dominant gene and the character, i.e., intestinal polyposis condition."

REFERENCES

1. *Cripps, H.:* Two cases of disseminated polypus of rectum. Trans. path. Soc., London *22*, 165, 1882.
2. *Dukes, C. E.:* Hereditary factors in polyposis intestini, or multiple adenomata. Cancer Rev. *5*, 241, 1930.
3. *Dukes, C. E.:* Familial intestinal polyposis. Ann. Eugen. *17*, 1, 1952.
4. *Gardner, E. J.:* A genetic and clinical study of intestinal polyposis, a predisposing factor for carcinoma of the colon and rectum. Amer. J. hum. Genet. *3*, 175, 1951.
5. *Gardner, E. J.:* Mendelian pattern of dominant inheritance for a syndrome including intestinal polyposis, osteomas, fibromas and sebaceous cysts in a human family group. Novant'anni delle Leggi Mendeliane ed. L. Gedda. Istituto G. Mendel, p. 321, Rome 1956.
6. *Leuchtenberger, C.:* Cytoplasmic "inclusion bodies" containing desoxyribose nucleic acid (DNA) in cells of human rectal polyps. Lab. Invest. *3*, 132, 1954.
7. *Leuchtenberger, C., P. F. Doolin* and *A. H. Kutsakis:* Buffered osmium tetroxide (OsO_4) fixation for cytological and Feulgen microspectrophotometric studies of human rectal polyps. J. Biophys. & Biochem. Cytol. *1*, 385, 1955.
8. *Leuchtenberger, C., R. Leuchtenberger* and *A. M. Davis:* A microspectrophotometric study of the desoxyribose nucleic acid (DNA) content in cells of normal and malignant human tissues. Am. J. Path. *30*, 65, 1954.
9. *Lockhart-Mummery, J. P.:* Cancer and Heredity. Lancet *1925/I*, 427.
10. *McCarty, R. T.:* The genetic factor of familial disseminated polyposis coli. Amer. J. Surg. *86*, 500, 1953.
11. *Miescher, F.:* Histochemischen und Physiologischen Arbeiten. Vogel, Leipzig 1897.
12. *Palade, G. E.:* A study of fixation for electron microscopy. J. exp. Med. *95*, 285, 1952.
13. *Toolan, H. W.:* Growth of human tumors in cortisone-treated laboratory animals; the possibility of obtaining permanently transplantable human tumors. Cancer Res. *13*, 389, 1953.

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ACTUALITÉS EN MATIÈRE D'ONCOGÉNÉTIQUE HUMAINE[1]

Par L. GIANFERRARI

Notre Institut a entrepris, depuis quelques années de recherches pour établir s'il existe réellement une concentration familiale des affections cancéreuses.

Nous avons considéré séparément les leucémies, les néoplasies du sein, de l'utérus, de la prostate et de la vessie.

Les néoplasies de l'estomac et du poumon sont en cours d'investigation. Il nous a paru intéressant de confronter les résultats déja acquis, comprenant par là une réélaboration parallèle de nos données et de celles qui avaient été publiées pendant ces derniers dix ans par d'autres auteurs et qui répondaient aux exigences d'une telle comparaison.

Pour les *leucémies* nous avons pris en considération les recherches de *Videbæk* [1947], de *Guasch* [1954], de *Morganti* et *Cresseri* [1954] et nous sommes parvenus à la conclusion qu'il existe un caractère familial pour les néoplasies dans l'ensemble, phénomène qui est dû surtout à la composante hétérotopique tandis qu'une composante homotopique est évidente seulement d'après les données de *Videbæk*.

Pour les *néoplasies du sein*, en considérant les recherches de *Jacobsen* [1946] et celles de *Bucalossi*, *Veronesi* et *Pandolfi* [1954] on peut admettre qu'il existe un caractère familial pour tous les sièges dans l'ensemble, ce qui est dû soit à la présence d'une composante homotopique, soit à la présence d'une composante hétérotopique, la première étant plus marquée que la seconde.

Pour évaluer ces résultats, il faut tenir compte aussi des travaux d'autres auteurs qui ont recherché l'existence d'un caractère familial,

[1] Le travail «in extenso» paraîtra dans « Acta Geneticae Medicae et Gemellologiae».

en se servant d'autres méthodes. Ce sont, en particulier, les cas de *Penrose*, *Mackenzie* et *Karn* [1947] qui ont démontré l'existence d'un caractère familial homotopique mais non hétérotopique du cancer du sein en se servant pour leur comparaison de valeurs établies sur base de la mortalité dûe au cancer dans la population anglaise.

Cependant, *Passey*, *Wainman*, *Amstrong* et *Rhodes* [1952] en limitant leur recherche aux femmes et en utilisant pour leur comparaison la même méthode, nient tout caractère familial du cancer du sein.

Pour les *néoplasies du corps de l'utérus*, d'après les données de *Brøbeck* [1949] et celles de *Beolchini*, *Cresseri*, *Gianferrari*, *Malcovati* et *Morganti* [1956] on peut admettre qu'il existe un caractère familial pour les néoplasies de tous les sièges dans l'ensemble plus marquée chez les hommes que chez les femmes, pour lesquelles le phénomène est dû aussi à une certaine tendance homotopique, à tel point que, limitant le parallèle à l'incidence des néoplasies des autres sièges, la différence de comportement entre les deux sexes devient encore plus évidente.

De telles conclusions concordent bien avec celles auxquelles s'est rallié *Brøbeck* en employant comme terme de comparaison les valeurs établies sur base de la mortalité dûe au cancer dans la population danoise.

En considérant les recherches de *Brøbeck* [1949], de *Murphy* [1950], de *Beolchini*, *Cresseri*, *Gianferrari*, *Malcovati* et *Morganti* [1956] pour les *néoplasies du col de l'utérus*, nous sommes parvenus à la conclusion que tandis que, d'après les données de *Brøbeck* et de *Beolchini* et coll., on démontre l'existence d'aucun caractère familial, d'après celles de *Murphy*, il reste un doute quant à l'existence d'une certaine tendance homotopique.

Pour les *néoplasies de la prostate*, les recherches originales de *Gianferrari*, *Arrigoni*, *Cresseri*, *Lovati* et *Morganti* [1956] ont servi de base et on est parvenu à la conclusion, qu'il existe un caractère familial seulement pour les néoplasies du même siège.

Pour les *néoplasies de la vessie*, d'après les recherches de *Gianferrari*, *Arrigoni*, *Cresseri*, *Lovati* et *Morganti* [1955], on peut admettre qu'il n'existe aucun caractère familial.

D'après les recherches de *Videbæk* [1954] on peut admettre que pour les *néoplasies de l'estomac*, il existe un caractère familial bien net pour les néoplasies du même siège, mais non pour celles des autres sièges.

Conclusions générales

On peut donc conclure à l'existence d'un caractère familial des néoplasies.

Il est évident sous l'aspect homotopique et hétérotopique pour les néoplasies du sein.

Pour les néoplasies de l'estomac et de la prostate, la composante homotopique est évidente, tandis que pour les néoplasies du corps de l'utérus et pour les leucémies, la composante hétérotopique prévaudrait.

Un doute subsiste quant à l'existence d'une composante homotopique pour les néoplasies du col de l'utérus.

Enfin, aucun caractère familial n'a pu être démontré pour les néoplasies de la vessie.

Des recherches basées sur l'étude du «cancer à deux» ou l'étude des jumeaux pourraient établir si un tel caractère familial est sous l'influence, comme il semble probable, de facteur idiotypiques.[1]

Discussion:

J. Clemmesen (Copenhagen): From the earliest stages of the organization of the Danish Cancer Registry an effort was taken to establish a collaboration with the University Institute for Human Genetics. As a result the monographs by *Jacobsen* [1946] on heredity in breast cancer and by *Brøbeck* [1949] on uterine cancer as well as later publications appeared. Through reassessments of the results made notably by *Busk* (Ann. Eugenics 1948, vol. 14, 213), *Clemmesen* (Brit. J. Cancer 1949, *3*, 474), and *Busk* (Proc. Sec. Nat. Cancer Conf., Cincinnati 1952, II, 1087), we have arrived at the same conclusions as Prof. *Gianferrari* that there is a slight tendency to inheritance of breast cancer. Relatives of women with cancer in the right breast stand a higher risk of aquiring cancer in the right than in the left breast contrary to other women. It also appeared from *Brøbeck*'s study that there is some tendency to inheritance of cancer of the uterine corpus, but not of the cervix. These results have been reached on the basis of information collected by the Cancer Registry on the social distribution of various malignant diseases combined with knowledge of the age distribution. It may be added that we have also found that *Videbæk*'s material [1947] indicates that leucemia is not inherited according to *Busk* [1952].

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[1] Tout récemment *v. Verschuer* [1956] a publié les résultats de ses recherches sur les jumeaux cancéreux. Il n'a pas trouvé d'évidence pour une concordance plus élevée chez les jumeaux monozygotes; ceux-ci cependant, lorsqu'il y a concordance, concordent aussi, presque toujours, quant au siège du cancer. En considérant séparément les diverses localisations *v. Verschuer* parvient à admettre une composante héréditaire pour le cancer de l'estomac, mais non pour les cancers du sein et de l'utérus. Cette dernière conclusion ne paraît pas en accord avec la démonstration qui a été donnée de l'existence d'un caractère familial des néoplasies du sein et du corps de l'utérus et nous souhaitons que ces recherches soient poursuivies et étendues.

Cresseri, A., L. Gianferrari, P. Malcovati, G. Morganti and *P. E. Beolchini*: Acta genet. 6, 301–303, 1956

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GENETICAL RESEARCHES ON UTERINE CANCER[1]

By A. CRESSERI, L. GIANFERRARI, P. MALCOVATI
G. MORGANTI and P. E. BEOLCHINI

562 cases of uterine cancer were investigated, viz. 144 cases of cancer of the corpus and 418 cases of cancer of the cervix. The cases were collected during the years 1949–1953 in different hospitals in Milan.

The groups of the cancers of the corpus and of the cervix were compared in respect of different data: statistically significant differences have been observed with regard to the age distribution (average age at onset: cancer of the corpus 56.3 ± 0.6 and cancer of the cervix 49.4 ± 0.5 years), the histological type (cancer of the corpus 93% adenocarcinomata, cancer of the cervix 91% squamous cell carcinomata) and the marital status (higher incidence of unmarried in the group of cancer of the cervix). On the contrary no difference was observed with regard to the number of conceptions, the number of liveborn and stillborn children, the number of abortions, the age at onset of menses and at onset of menopause and the age at last conception.

The genetical investigation was undertaken in order to ascertain the presence of cancers of all types and sites in some categories of relatives (father, mother, grandfather, grandmother, uncles, aunts, brothers, sisters, sons and daughters). It was not possible for all the 144 patients with cancer of the corpus uteri and for all the 418 patients with cancer of the cervix uteri, to collect the genetical data: the collection was possible only for 91 and 199 subjects respectively (probands). The proband groups may be considered representative samples of the corresponding patient groups, as no significant difference exists with regard to the year of hospitalization, the age at onset of the disease and the marital status.

[1] The work "in extenso" is to be published in "Acta Geneticae Medicae et Gemellologiae".

Cancer of the corpus uteri

Group	No	Relatives		Incidence of cancer								
				All sites			Same site			Other sites		
		Sex	No	No	%	P	No	%	P	No	%	P
Probands	91	♂	492	30	6.1	♂				30	6.1	♂
		♀	465	34	7.3	0.10 > P > 0.05	13	2.8		21	4.5	0.10 > P > 0.05
		♂+♀	957	64	6.7					51	5.3	
						♀			♀			♀
						0.50 > P > 0.30			P ≃ 0.03			P > 0.90
Controls	91	♂	453	15	3.3					15	3.3	
		♀	438	24	5.5	♂+♀	4	0.9		20	4.6	♂+♀
		♂+♀	891	39	4.4	0.05 > P > 0.02				35	3.9	0.20 > P > 0.10

Cancer of the cervix uteri

Group	No	Relatives		Incidence of cancer								
				All sites			Same site			Other sites		
		Sex	No	No	%	P	No	%	P	No	%	P
Probands	199	♂	1120	41	3.7	♂				41	3.7	♂
		♀	1071	46	4.3	0.70 > P > 0.50	9	0.8		37	3.4	0.70 > P > 0.50
		♂+♀	2191	87	4.0					78	3.5	
						♀			♀			♀
						0.80 > P > 0.70			P > 0.90			0.70 > P > 0.50
Controls	199	♂	1232	39	3.2					39	3.2	
		♀	1127	53	4.7	♂+♀	9	0.8		44	3.9	♂+♀
		♂+♀	2359	92	3.9	P > 0.90				83	3.5	P > 0.90

A control material was collected in a similar way, taking as probands corresponding subjects without cancer. Significant differences are not shown by the comparison of the age distributions of the different categories of relatives in the proband and control groups: therefore the control material may be considered suitable to be compared with the proband material, with regard to the incidence of cancer of all sites, the same site (without distinction between cancer of the corpus and cancer of the cervix uteri) and other sites (see the table).

A relative excess of families with one or more cases of cancers (of all sites) is observed only in the group of cancer of the corpus uteri.

As it is shown in the table (P has been calculated by means of a χ^2 test or by means of the exact treatment for 2×2 tables) significant or nearly significant differences of the relative incidence of cancers between the families of the probands and the families of the corresponding controls, have been observed only in the group of cancers of the corpus uteri, namely for the cancers of all sites (males and females together) and for the cancer of the same site.

No significant difference exists in the group of cancer of the cervix uteri.

In conclusion, our data show a significant familial incidence for the cancers of all sites and for the cancer of the same site only for the cancer of the corpus and not for the cancer of the cervix uteri.

Morganti, G., L. Gianferrari, A. Cresseri, G. Arrigoni et *G. Lovati*: Acta genet. *6*, 304–305, 1956

Istituto di Genetica dei tumori «Cassa di Risparmio PP. LL.»
presso il Centro di Studi di Genetica Umana, Università di Milano, Italia

RECHERCHES CLINICO-STATISTIQUES ET GÉNÉTIQUES SUR LES NÉOPLASIES DE LA PROSTATE[1]

Par G. MORGANTI, L. GIANFERRARI, A. CRESSERI, G. ARRIGONI et G. LOVATI

On considère ici 265 malades du cancer de la prostate qui furent hospitalisés entre 1939 et 1954 à la Clinique Urologique de l'Université de Milan.

Au point de vue clinico-statistique, on a étudié la distribution de ce matériel selon l'âge au diagnostic (âge d'incidence maxima entre 65 et 69 ans), le métier (pas de corrélation), la survie (moins de 50% deux ans après le diagnostic), la présence et le degré des divers symptômes (conditions générales, pression artérielle, azotémie, hématurie, dysurie, cystite, rétention, perméabilité urétrale, capacité et aspect cystoscopique de la vessie, sédiment urinaire, caractères de la prostate).

Ce n'est que pour 183 des 265 patients qu'on a pu obtenir des données d'état civil et cliniques suffisantes pour les parents, grands-parents, oncles, frères, fils. Nous avons contrôlé que dans la constitution de ce groupe de 183 probants il n'y ait pas eu de sélection à l'égard du matériel de départ constitué par les patients. Ceci a pu se démontrer soit pour la distribution selon l'année d'hospitalisation soit pour la distribution selon l'âge.

Pour pouvoir étudier l'incidence de néoplasies de tous les sièges et en particulier de la prostate dans les consanguins des probants on a récolté, avec des critères analogues à ceux qu'on avait suivis pour les consanguins des probants, les données d'état civil et cliniques se référant aux consanguins de 183 sujets sains. La correspondance des deux matériels a été vérifiée en comparant les distributions selon l'âge des différentes catégories de consanguins des probants et des témoins.

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[1] Le travail «in extenso» est publié dans «Acta Geneticae Medicae et Gemellologiae», 5, 224–233, 1956.

En classant les familles des probants et des témoins selon le nombre des néoplasies trouvées dans chacune d'elles nous avons obtenu les données rapportées dans le tableau n° 1, qui démontrent, sans toutefois des différences significatives, une incidence relativement plus grande de familles sans cas de néoplasies pour les témoins et de familles avec un ou plusieurs cas pour les probants.

Tableau n° 1

Catégorie des familles	Familles des probants	Familles des témoins
Sans cas de néoplasies	122	132
Avec 1 cas de néoplasie	40	34
Avec 2 cas de néoplasies	12	10
Avec 3 cas de néoplasies	7	6
Avec 4 cas de néoplasies et plus	2	1
Total	183	183

L'incidence de néoplasies dans la totalité des consanguins des probants et des témoins est rassemblée dans le tableau n° 2. Pour les néoplasies de tous les sièges dans l'ensemble on n'atteint pas de différences significatives ni pour la totalité ni pour les deux sexes considérés séparément. De même, on n'observe pas de différences significatives si on considère les incidences des néoplasies de tous les sièges à l'exclusion de celles de la prostate. Par contre, le caractère familial ressort avec une signification élevée si on observe seulement les néoplasies de la prostate. L'existence d'une composante homotopique indépendante est confirmée par la comparaison de l'incrément de l'incidence des néoplasies de la prostate avec l'incrément de l'incidence des néoplasies dans l'ensemble.

En conclusion on peut admettre que pour les néoplasies de la prostate un caractère familial est démontrable seulement pour les néoplasies du même siège.

Tableau n° 2

Siège des néoplasies	Consanguins des probants	Consanguins des témoins
Appareil gastro-entérique et glandes annexes	48	34
Appareil génito-mammaire	12	14
Prostate	11	1
Autres sièges	22	27
Total	93	76

Morganti, G., J. Gianferrari, A. Cresseri, G. Arrigoni et G. Lovati: Acta genet. *6*, 306–307, 1956

Istituto di Genetica dei Tumori «Cassa di Risparmio PP.LL.»
presso il Centro di Studi di Genetica Umana, Università di Milano, Italia

RECHERCHES CLINICO-STATISTIQUES ET GÉNÉTIQUES SUR LES NÉOPLASIES DE LA VESSIE[1]

Par G. MORGANTI, L. GIANFERRARI, A. CRESSERI, G. ARRIGONI et G. LOVATI

On considère ici 277 malades du cancer de la vessie qui furent hospitalisés entre 1939 et 1954 à la Clinique Urologique de l'Université de Milan.

Au point de vue clinico-statistique on a étudié la distribution de ce matériel selon l'âge au diagnostic (âge d'incidence maxima entre 60 et 64 ans), le sexe (72,9% de mâles), le métier (5,5% d'ouvriers de l'industrie chimique), la survie (moins de 50% un an après le diagnostic), la présence et le degré de divers symptômes (conditions générales, pression artérielle, azotémie, hématurie, dysurie, cystite, perméabilité urétrale, capacité de la vessie, rétention, caractères de la prostate, aspect cystoscopique et cystographique de la vessie, siège et caractères de la néoplasie).

Ce n'est que pour 160 des 277 patients qu'on a pu obtenir des données d'état civil et cliniques suffisantes pour les parents, grands-parents, oncles, frères, fils. Nous avons contrôlé que dans la constitution de ce groupe de 160 probants il n'y ait pas eu de sélection à l'égard du matériel de départ constitué par les patients, ce qui a pu se démontrer pour la distribution selon l'année d'hospitalisation, selon l'âge, selon le sexe et selon le métier.

Pour pouvoir étudier l'incidence de néoplasies de tous les sièges et en particulier de la vessie dans les consanguins des probants on a récolté,

[1] Le travail «in extenso» est publié dans «Novant' anni delle leggi mendeliane» par L. Gedda, Editions de l'Institut *Gregorio Mendel*, Rome, 340–350, 1956.

Tableau n° 1

Catégorie des familles	Familles des probants	Familles des témoins
Sans cas de néoplasies	97	106
Avec 1 cas de néoplasie	42	38
Avec 2 cas de néoplasies	15	11
Avec 3 cas de néoplasies et plus	6	5
Total	160	160

avec des critères analogues à ceux qu'on avait suivis pour les consanguins des probants, les données d'état civil et cliniques se référant aux consanguins de 160 sujets sains. La correspondence des deux matériels a été vérifiée en comparant les distributions selon l'âge des différentes catégories de consanguins des probants et des témoins.

En classant les familles des probants et des témoins selon le nombre des néoplasies trouvées dans chacune d'elles nous avons obtenu les données rapportées dans le tableau n° 1, qui montrent l'absence de différences appréciables.

Tableau n° 2

Siège des néoplasies	Consanguins des probants	Consanguins des témoins
Appareil gastro-entérique et glandes annexes	36	31
Appareil génito-mammaire	16	14
Vessie	4	2
Autres sièges	38	28
Total	94	75

L'incidence de néoplasies dans la totalité des consanguins des probants et des témoins est rassemblée dans le tableau n° 2. L'analyse des données montre, pour la totalité et pour les deux sexes considérés séparément, l'absence de différences significatives, soit pour les néoplasies dans l'ensemble, soit pour les néoplasies de la vessie, soit pour les néoplasies des autres sièges.

En conclusion on doit admettre que pour les néoplasies de la vessie un caractère familial n'est pas démontrable.

Peller, S.: Acta genet. *6*, 308–314 1956

GENETICS OF CHILDHOOD CANCER

By S. PELLER, New York, U.S.A.

The question of heredity of human cancer is old. *K. Pearson* [1902], sought the answer in *family surveys*. *Bashford* [1904] rejected this method on two grounds:

1. lack of diagnostic reliability.
2. for chance variations alone—the whole array of combinations from cancer-free to cancer-rich families is to be expected. In itself an example of a family with 10 or more cases of common forms of cancer is no evidence of heredity.

In the last 50 years, family surveys have been rather frequently used. I mention the Norwegian studies from *Soegård* to *Waaler*, *Roger Williams*, *Levin*, *Warthin* and, more recently, *Wassink*, *Hauser* and *Weller*, *Jacobsen*, *Bargen* and collaborators, *Smithers*, *Brøbeck*, *Videbæk*, *Murphy*, *Hagy* and *Woolf*. Most of the authors believed to have established or confirmed heredity of cancer, especially organ disposition to cancer which, according to *Levin* [1912], is a *dominant* trait. *Bauer* and *Aschner* [1922] looked at the problem differently, assuming two genes in operation, one responsible for the disposition to, the other for the localization of, cancer.

The other method used in studies on heredity of cancer is that of *twins*. I mention *Macklin*, *Habs*, *von Verschuer* and *Kober*, *Busk*, *Clemmesen* and *Nielsen*, and *Gorer*. According to *Macklin*, genetic constitution determines the general disposition, the choice of the organ, the structure of the tumor and the age of its appearance. As you know, many forms of cancer—common and rare—predominate in one sex. Has this fact been given proper consideration? The predominance in one sex is reason enough for cancer to be more frequent in cotwins of propositi in homozygotic and like-sexed fraternal twins than in unlike-sex twin pairs.

So far the cancer problem has not been clarified by the collection of family data or by the twin studies. I agree with *Curt Stern*'s statement, [1949], that the genetics of cancer is still very incompletely understood. The two methods, discussed above, have only indicated that heredity

may play some role in the origin of cancer. The question is: where and how does heredity come into play?

My observations on human cancer since 1921, based on clinical, hospital and postmortem material, on surveys in miners, the American Armed Forces, and on mortality statistics, revealed a high variability of cancer distribution by site and of total cancer mortality in the population. As a rule, close to 20 per cent of adults have cancer. Under special conditions, for instance in radium miners, up to 50 per cent develop cancer. The incidence of cancer in one organ, such as the lungs, can increase 25–29 times above the contemporary average, and probably more. In such a population sample up to 90 percent of all primary cancers are localized in one organ or system, and only 10 per cent are scattered over the other organs. The latter have then a much smaller incidence than normal. Physiological processes, such as reproductive activity, and many extrinsic factors, such as sun and Xrays, radium emanation, tar, anilin, burns etc., are apt to change and do change the organ distribution of cancer in a population. My conclusion from these studies—as summarized in my book "Cancer in Man", 1952—is: whether under given circumstances an adult develops cancer depends on *the degree* of his disposition which reflects his genetic make-up, but the spot and organ as well as the time of cancer appearance is *not* genetically determined.

The organ distribution and histogenesis of cancer in children are entirely different from those in adults. Moreover, children have some cancer forms hardly ever encountered in adults, and they have a cancer age curve that rises only for about three to four years and falls the following ten years. This triad of deviations was never satisfactorily explained.

Are these deviations genetically conditioned?

A satisfactory explanation of these features of childhood cancer becomes feasible if two principles are logically applied to it:

a) there is a latency period between initiation and manifestation of cancer;

b) the majority of cancers arise in tissues which are in contact with the outer world and are first acted upon and stimulated by the extrinsic cancerogens; and if we realize that

c) as far as cancerogenesis goes on in the fetus, it is associated with a blood circulation that differs in many respects from the circulation post partum.

Cancer frequency is relatively high in infancy. As cancer is preceded by a latent period, the initiation occurs in the fetus. Cancerogens mostly come from outside. Just as oxygen and nutrients they can enter the

embryo and fetus via placenta only (except X-rays), and within the fetal body have the same distributor: the circulatory organs. With one stroke the anatomy and physiology of blood circulation reveal the reason for the main peculiarities of organ distribution of childhood cancers, as far as they were initiated intrafetally. The peak of the age of childhood cancer is at three (to four) years. This pinpoints the birth as the end of the period in which childhood cancers were initiated.

We have now a logical explanation for:

1. complete absence of cancer in aborted embryos and young fetuses (*Flanagan*),
2. the development of some cancers in older fetuses,
3. the high incidence of cancer in infants and young children,
4. the decline of cancer incidence in children 4 to 14 years of age,
5. the tremendous ratio of leukemia among childhood cancers,
6. the high ratio of brain and eye cancers,
7. the low incidence and ratio of cancers in the digestive organs,
8. the peculiar distribution of cancers within the digestive organs,
9. the rarity of bladder cancers as compared with kidney cancers,
10. the high ratio of sarcomas, etc., etc.

Cancerous mothers seldom give birth to a cancerous child, and most cancerous children have healthy parents. Some children of mothers with cervix cancer develop leukemia, some children of leukemic mothers may develop retinoblastoma and so on. We are not surprised by such variations. They imply that all primary cancers—regardless of the organ of origin and histology—are due to the action of cancerogens upon an individual with a genetic make up which accounts for the degree of the disposition to cancer but *not* for the localization and structure of the tumor.

My findings are incompatible with the idea of an organ disposition to cancer. Nevertheless, in cancer of some organs and in some forms of cancer, organ-specific factors sometimes play a role. These factors either help to increase the amount of cancerogens brought to bear upon that organ or they diminish its resistance to cancerogens. The effectiveness of such organ-specific factors in determining the outcome of cancerogenesis varies from absolute to negligeable. A few examples pertaining to childhood cancer may elucidate this range of interference.

1. *Anomalies having a decisive influence* upon the age, the clinical appearance and gravity of cancer and its familial agglomeration.

The rare form of skin cancer called *xeroderma cancer* is impossible without a skin anomaly which is due to an incompletely sex linked reces-

sive defective gene. There also exist skin carcinomas, sarcomas and melanomas in children, *not* associated with this skin anomaly. All skin cancers in children are due to the same intrafetal happenings, namely saturation of parts of the skin with cancerogens distributed in cancer-disposed fetuses in accordance with the normal blood circulation. Sun rays are only an additional, a non-essential, cancer-provocative factor which speeds up and aggravates, but does not create the xeroderma cancers. For the latter, the combination of:

a) the hereditary skin anomaly,

b) the general disposition to cancer, and

c) the intrafetal cancerogenesis is essential, the skin anomaly *dominating* the age, the clinical picture and the outcome, but *not* determining the part of the skin in which the xeroderma becomes manifest.

2. *Anomalies influencing familial accumulation only: Retinoblastoma* is practically the only eye cancer encountered in childhood. Most cases are sporadic, a few percent show agglomeration in families. This accumulation, according to *Weller*, greatly exceeds chance. What is the reason for the accumulation of the tumor in some families? According to *Bell*, *Hine*, *Franceschetti* and *Sorsby* retinoblastoma is an *autosomal dominant affection*—although it frequently skips a generation and only seldom has been observed in three generations in succession. The mutation rate is 1.4×10^{-5} according to *Philip* and *Sorsby*, 2.3×10^{-5} according to *Neel* and *Falls*, and 4.4×10^{-6} according to *Vogel*. I deny validity to the theory and to the calculations. *Hemmer*, *Reiser* and *Keller* rejected the hereditary hypothesis altogether.

To understand retinoblastoma we should know:

a) that this tumor develops only in the pars *optica* retinae, not in the pars ciliaris or in the pars iridica retinae;

b) that the child's lens, supplied by the same hyaloid artery, is free of tumors;

c) that the embryonal and fetal optic retina manages to develop although for ten or eleven early weeks it is completely avascular and isolated from the blood vessels;

d) that it is nevertheless well supplied with O_2, nutrients and apparently also with cancerogens.

All this is made possible by a peculiar vascular arrangement in the vitreous, where the hyaloidea capillaries are not thoroughfares—as in the lens capsule etc.—but saccular end-capillaries. The hyaloid artery supplies the whole retina and lens, but on account of the hyaloid capillaries in the vitreous, a large part of O_2, nutrients and cancerogens, which otherwise would be distributed between optic retina, ciliary retina, iridical retina and the lens, go to the optic retina. Thus the optic retina gets sometimes fully saturated with cancerogens, and tumefaction starts. The other parts of the retina and the lens remain unsaturated and therefore free of cancer.

Lack of cancers in the lens is *not* a genetic characteristic but is due to the insufficient amount of cancerogens. This was experimentally proven by *Ida Mann*. With the collapse of the hyaloid circulatory system and the development of circulatory conditions in the eye as they persist after birth, the reasons for the promotion of cancerogenesis in the optic retina cease to exist. In adults, therefore, the distribution of cancers in the eye is different from that in childhood.

I have just explained the topography of sporadic childhood cancers in the eye. They are due to a *somatic cell mutation in the optic retina of a cancer disposed fetus*. How are now the cases of familial agglomeration to be explained? In *these* families, obviously, there exist some promoting factors. They increase the chance of satisfactory accumulation of cancerogens in the eye in from two to ten family members. As promoting factors—I have in mind—anomalies apt either to *increase the amount* of cancerogens in the embryonal and early fetal optic retina or to *diminish its resistance* to cancerogenic action. *Von Hippel*'s experiments on rabbits proved the hereditary character of retinal and choroidal colobomas. If the blood of the ophthalmica happens to carry cancerogens and there are defects in both the pigment epithelium and *Bruch*'s membrane, then the retina receives cancerogens not only via vitreous from the hyaloidea but also from the choroid, even if also the choriocapillaris is defective.

More than one of the siblings have a chance of developing retinoblastoma, if several siblings happen to have coloboma—which might be a *dominant monofactorial* characteristic—and the maternal blood was in all respective pregnancies rich in cancerogens.

An effect similar to that in coloboma may result from an unusually prolonged life of the saccular endcapillaries, or from anomalies of the hyaloidea that hinder formation of the second vitreous and cause retinal folds leading to a rupture of the membrana limitans interna. Hence can-

cerogens gain free access to undifferentiated retina cells which now are *not* protected by the membrana limitans. The unprotected retinal cells react with wild proliferation and rosette formation. (These folds are usually a hereditary anomaly; they can also be produced by X-ray exposure of the fetus.) Slowed down retinal differentiation or anterior extension of the optic retina might also promote initiation of retinoblastoma. *Each of the anomalies* which promote accumulation of cancerogens in the retina may have *its genetically fixed transmission pattern*, but retinoblastoma is not transmitted.

Thus, a *sporadic* retinoblastoma depends on the coincidence of two factors, namely,

a) the sufficient amount of maternal cancerogens in the ophthalmic artery and,

b) the general cancer disposition of the fetus.

A familial retinoblastoma needs also a third factor, namely,

c) one of the mentioned hereditary anomalies which have different transmission patterns.

The first two points are essential, the third only *promotes* initiation of retinoblastoma. Even if both parents carry a certain dominant eye anomaly, as a rule not all of their children but some or none are going to develop the tumor.

Coloboma and other anomalies persist after birth and do not kill the carrier. However, the fetal circulatory conditions are replaced at birth. Moreover, most of the cells already are differentiated. Therefore, the probability of postnatal creation of retinoblastoma drops to a point close to zero. (There are other hereditary disturbances in the eye, such as abiotrophies and phakomatoses which manifest themselves only after birth. Hence, they are too late to play a role in promoting retinoblastoma.)

3. *Cancers in which no hereditary organ-specific promoting factors are involved:* The third form of specific childhood cancer, the *sympathoneuroblastoma* of the adrenal medulla and paraganglia shows no hereditary trait at all. Small amounts of cancerogens as they are carried in abdominal arteries, augmented by the products of destruction of the huge mass called "fetal adrenal cortex" act upon the ganglion cells of the adrenal medulla and the paraganglia. These are the only ganglion cells in the body which normally are in direct contact with blood capillaries. Therefore, there are more ganglion cell tumors in the little adrenal medulla or paraganglia than are found in the whole central nervous system, although

the cancerogen content of the blood in the brain and upper spine is much higher than in the adrenal arteries.

Organ specific promoting factors are not limited to the discussed forms of cancer. They may and they do exist also in other organs, whether we know them or not. Such promoting, and therefore cancerrelevant factors explain familial occurrence of some cancers in adults. Thus, of breast or stomach cancer or of leukemia a considerable percentage of cases *may* show accumulation in some families, although cancer disposition is not organ linked. However, not all anomalies are cancerrelevant. An example is spina bifida which is not cancerrelevant at all.

Conclusion

In all forms of cancer in man there is always an *environmental* factor, cancerogens, that causes a somatic cell mutation, and always an *autosomal* factor, that is the *general* disposition. The former factor explains the high variability of the organ distribution and structure of cancer. The disposition to cancer is *not* organ-specific, but in some forms of human cancer promoting organ-bound factors, like anomalies, either hereditary—*sex linked or autosomal*—or sporadic, sometimes become cancerrelevant.

Nielsen, A. and *J. Clemmesen:* Acta genet. *6*, 315–316, 1956

The Danish Cancer Registry under the National Anti-Cancer League,
Copenhagen, Denmark

TWIN STUDIES IN THE DANISH CANCER REGISTRY

By A. NIELSEN and J. CLEMMESEN

Summary

During the years from January 1942 to July 1955 ca. 140.000 fresh cases of malignant diseases were notified to the Danish Cancer Registry, 110.000 of which cases were notified from hospitals. In most of the latter cases it was stated whether or not the patient was a twin, and thus 849 pairs were notified.

Twin pairs were grouped according to reliability of information on cancer, and on similarity in appearance. In 80 per cent of cases information was considered sufficient, but in $^1/_3$ of these one twin had died young or emigrated.

Two methods of study were employed: the "retrospective" method according to which the past history of both partners was examined on the notification of the first of them, and the "prospective" method which means that the period of observation of both partners takes its beginning when the first partner is notified. Computation of the number of cases of malignant disease to be expected according to age distribution and period of observation showed that the "prospective" method was far more reliable than the "retrospective", which reduced the number of pairs fit for study to 336.

In this limited material the authors found features suggestive of a feeble hereditary tendency in neoplastic diseases. This tendency, however, is not statistically significant and seems fully explained by the presence of such neoplastic diseases as are known from other studies to be genetically influenced.

Discussion:

S. Peller (New York): Unfortunately family studies still suffer from lack of diagnostic reliability. Moreover one has to consider the interpretation. It is doubtless that only some cancers show familial accumulation. There is no proof that the latter is due to genetics of cancer itself. Other factors may be involved, such as certain hereditary anomalies of the respective organ or system. They may be only *indirectly* cancer-relevant.

As to the twin method, its results, so far, have been disappointing. I have pointed this out years ago [1952] and it also was ascertained to day in the Plenary Session. Imagine that for a disease as easily recognizable as retinoblastoma, known for almost 200 years and known to be familial for about 150 years—so far only 10 pairs of twins have been collected. The twin method is potentially sound, but up to the present time it was too haphazardly used to be of value. Also with regard to twin studies the question of interpretation of results must be stressed. There is no scientific basis for the conclusion that it is cancer itself which is hereditary; it may be that the accompanying anatomical or physiological anomalies cause concordance.

We have to study pathological embryology in order to understand familial accumulation of cancer just as we have to recourse to physiological embryology in order to comprehend sporadic cancer of childhood.

J. Clemmesen (Copenhagen): As an answer to Dr. *Peller* I would like to say that we have in this paper only been concerned about whether our results showed differences. We have found it premature to discuss causes as long as we do not have differences which are statistically significant.

S. KARGER AG BASEL / NEW YORK

Prof. Dr. E. LUDWIG
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